Biomedical Research Advisory Council

Bankhead-Coley Cancer Research Program
James and Esther King Biomedical Research Program
Live Like Bella Pediatric Cancer Research Initiative
Zika Research Grant Initiative

2017-2018 Annual Report

Rick Scott
Governor

Celeste Philip, MD, MPH
Surgeon General and Secretary of Health

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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 Bankhead-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) 2017-2018, 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. Research grants are issued based on a competitive peer-review process. Awards from the King and Bankhead-Coley Programs 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. In FY 2017-2018, the Legislature appropriated $16,257,714 which funded a total of 20 Bankhead-Coley and King grants.

On July 1, 2017, the Legislature appropriated $2 million in research funding as part of the Live Like Bella Pediatric Cancer Research Initiative. These funds were awarded to five 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.

In 2016, Governor Rick Scott authorized $25 million in state funds to support the Zika Research Grant Initiative (Zika Initiative). The intent of these research funds was to initiate new research and discoveries focused on Zika virus infection. In 2017, the 34 awarded Zika Initiative research grants were contractually terminated.

Per statute requirements, a 2017-2018 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; and
- 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 LIVE LIKE BELLA PEDIATRIC CANCER RESEARCH INITIATIVE

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

- Significantly expand pediatric cancer research capacity in Florida.
- Improve both research and treatment through greater pediatric enrollment in clinical trials networks.
- Reduce the impact of pediatric cancer on disparate groups.

THE ZIKA RESEARCH GRANT INITIATIVE

The Zika Initiative provides grants for research to pursue the following goals:

- Support the development, testing, or delivery of a vaccine or other methods to prevent Zika infection;
- Develop innovative, cost-effective Zika testing methods or therapeutics; and
- Investigate health impacts of Zika virus on children and adults.

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 2017, are listed below (Biographical Statements or Curriculum Vitae is 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

Susan Vadaparampil, Ph.D., MPH, Senior Member, Department of Health Outcomes and Behavior, Moffitt Cancer Center and Research Institute; Seat: Governor

Abubakr A. Bajwa M.D., FCCP, Division Chief, Associate Professor of Medicine, Medical Director Pulmonary Hypertension and Interstitial Lung Disease Clinic, Division of Pulmonary, Critical Care and Sleep Medicine, University of Florida College of Medicine; Seat: American Lung Association

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

Barbara A. Centeno, M.D., Senior Member and Director of Cytopathology and Anatomic Pathology Quality Assurance, Moffitt Cancer Center; Seat: House of Representatives

David A. Decker, M.D., Attending Physician, Orlando Veterans Administration Medical Center; Seat: Governor

New Members Appointed After July 1, 2017

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., MPH, 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 timeframes. 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 Department's commitment to transparency in communicating program priorities, defines the BRAC's substantive areas of focus, specifies timeframes 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 (INDs) applications or Investigational Device Exemptions (IDEs) 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).

Fiscal Year 2017-2018 funding cycle awards were made to support the following research priorities for Bankhead-Coley, King and Live Like Bella grants:

17 Awards – Prevention and Treatment: 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.

4 Awards – Technology Transfer Feasibility (TTF): The goals of the TTF grant mechanism are to stimulate technology transfer activities for promising research discoveries that could lead to innovations in the prevention, diagnosis, treatment, and/or cure of cancer and strengthen a project's economic feasibility and commercialization prospects.

2 Awards – Tobacco Use: Reduction of tobacco use in children, adolescents, and adults.

1 Award – Health Disparities: Research that contributes to reductions in deaths due to the cancers listed above resulting from health disparities due to race, ethnicity, or income.

0 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.

1 Award – 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, central nervous system, reproductive, developmental impairment, Graft-versus-host disease).

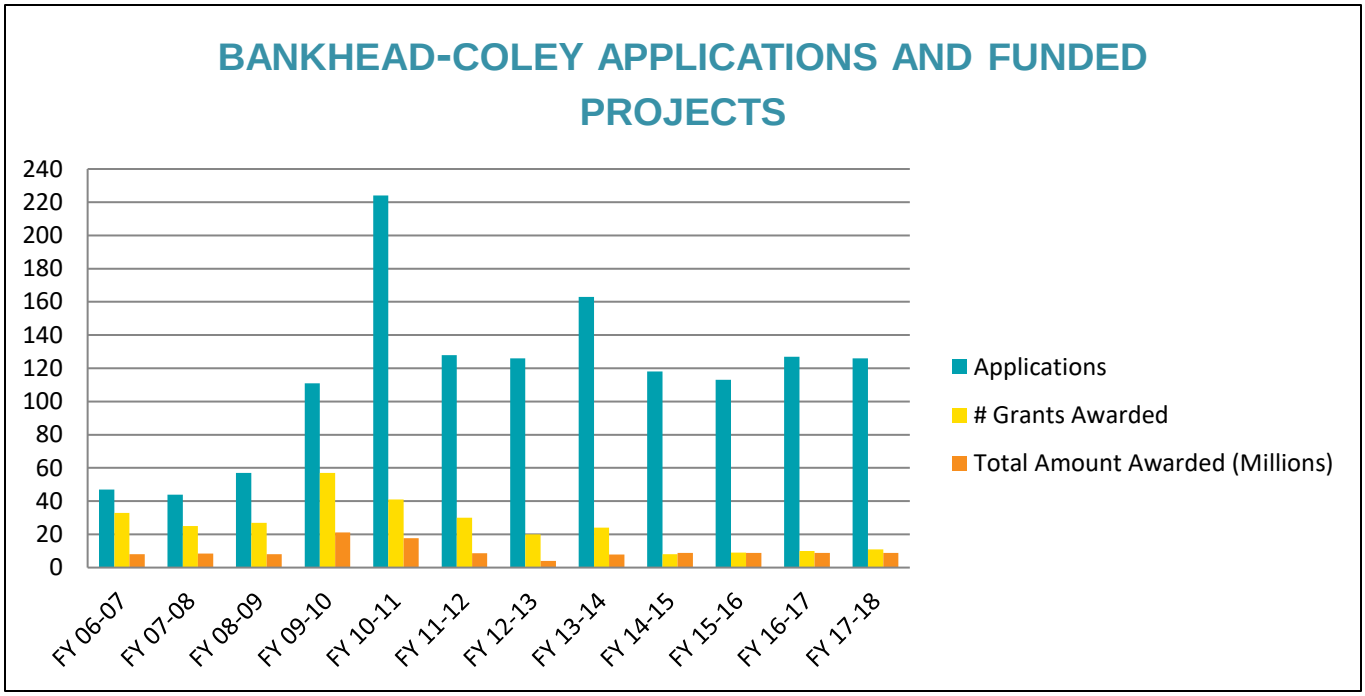

Figure 1: For FY 17-18, eleven projects were funded through Bankhead-Coley for cancer-related diseases. Eight were funded to support Prevention and Treatment; and three were funded for Technology Transfer Feasibility (TTF). A total of 126 grant applications were submitted for consideration of Bankhead-Coley funding.

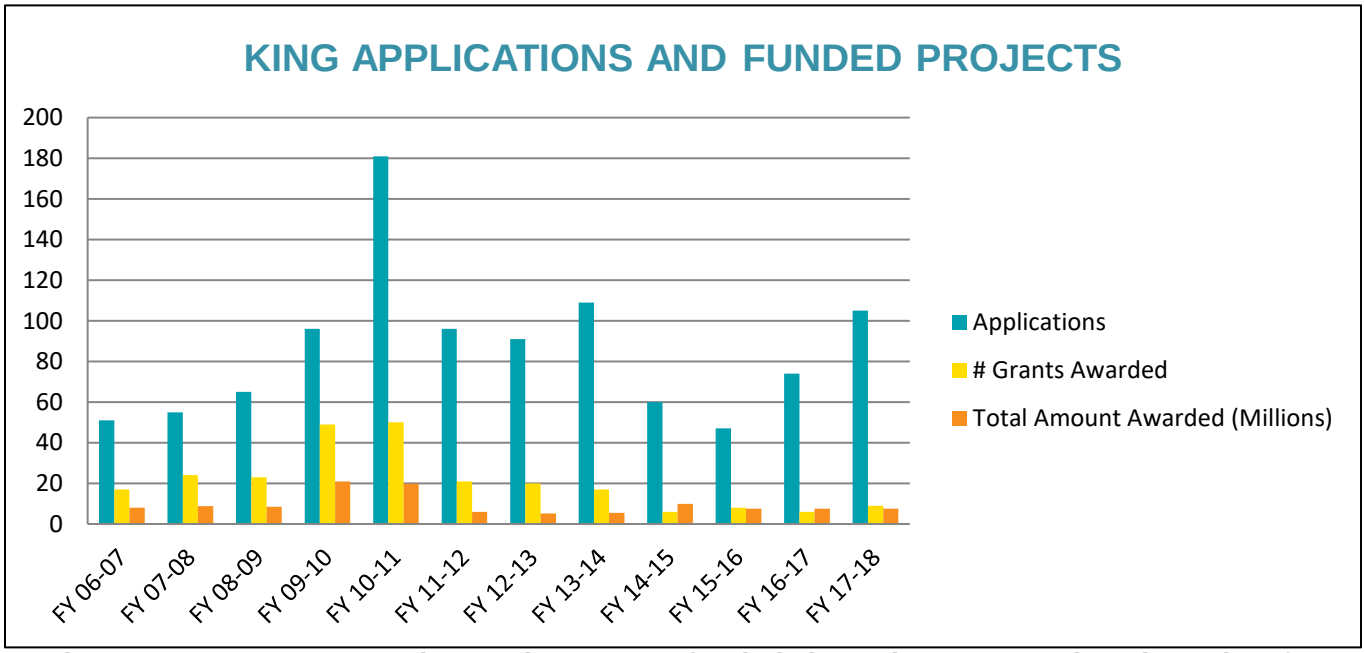

Figure 2: For FY 17-18, nine projects were funded through James and Esther King for Tobacco-related diseases. Seven were funded to support Prevention and Treatment; one was funded for Technology Transfer Feasibility (TTF); and one was funded for Health Disparities. A total of 105 grant applications were submitted for consideration of James & Ester King funding.

NATIONAL INSTITUTES OF HEALTH (NIH), RESEARCH FUNDING AND STATE RANKING

For the past three years, the state of Florida has remained twelfth in the United States for federal funding. While remaining in twelfth, there was an increase in the total amount of funding for FY 2017-2018.

National Institutes of Health Biomedical Research State Funding and Rankings Fiscal Year 2017

State	Total Funding	Rank
CA	$4,164,579,726	1
MA	$2,864,112,260	2
NY	$2,584,129,040	3
PA	$1,777,837,860	4
NC	$1,311,349,850	5
TX	$1,219,742,672	6
MD	$1,029,283,372	7
WA	$1,006,432,505	8
IL	$875,697,056	9
OH	$777,395,968	10
MI	$760,590,726	11
FL	$602,622,087	12
MO	$589,081,019	13
GA	$561,611,107	14
CT	$556,735,803	15
MN	$549,857,567	16
TN	$517,800,100	17
WI	$476,357,696	18
CO	$401,262,879	19
OR	$343,046,600	20

Figure 3: NIH Research Funding from the 2017 Fiscal Year Reporting Period. The top twenty states in NIH funding is displayed. With over $602 million in NIH funding, Florida is ranked 12th in the nation. *Source: NIH Research Portfolio Online Reporting Tools (RePORT)*

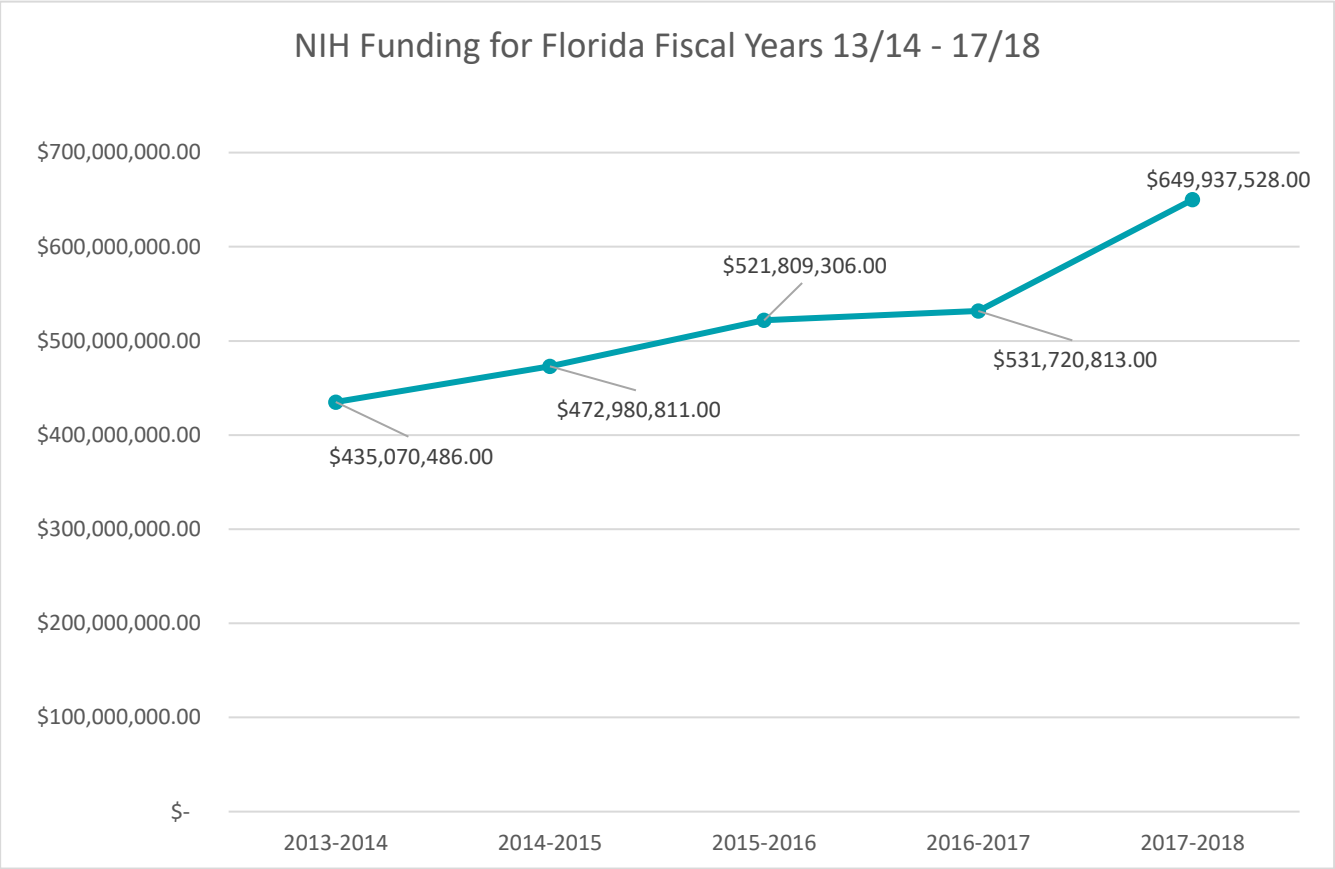

Figure 4: NIH Funding for Florida has increased in the last five years. These results reflect the Florida's initiative to expand upon research to improve scientific understanding of various diseases and health disparities.

Bankhead-Coley Cancer Biomedical Research Program

APPENDIX A

FISCAL YEAR 2017-2018 NEWLY AWARDED 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	$ 88,139.00	$ 727,144.00	5/01/2018	4/30/2021	No	No	No
8BC02	H. Lee Moffitt Cancer Center	Ruffell, Brian	$ 815,289	$ 67,940.00	$ 747,349.00	4/20/2018	3/31/2021	No	No	No
8BC03	H. Lee Moffitt Cancer Center	Smalley, Keiran	$ 815,283	$ 67,940.00	$ 747,343.00	4/02/2018	3/31/2021	No	No	No
8BC04	H. Lee Moffitt Cancer Center	Gillies, Robert	$ 815,283	$ 67,940.00	$ 747,343.00	3/21/2018	3/31/2021	No	No	No
8BC05	University of Central Florida	Phanstiel, Otto	$ 815,283	$ 67,940.25	$ 747,342.75	5/30/2018	3/31/2021	No	No	No
8BC06	University of Miami	Hudson, Barry	$ 815,283	$ 67,940.00	$ 747,343.00	4/20/2018	3/31/2021	No	No	No
8BC07	University of Miami	Saluja, Ashok	$ 815,282	$ 67,940.00	$ 747,342.00	4/04/2018	3/31/2021	No	No	No
8BC09	University of Miami	Wieder, Eric	$ 1,358,805	$ 75,490.00	$ 1,283,315.00	6/08/2018	3/31/2021	No	No	No
8BC10	University of Miami	Dhar, Shanta	$ 815,283	$ 67,940.25	$ 747,342.75	4/11/2018	3/31/2021	No	No	No
8BC11	South Florida Veterans Affairs Foundation	Perez-Stable, Carlos	$ 57,500	$ 28,750.00	$ 28,750.00	4/12/2018	9/30/2018	No	No	No
8BC12	Florida State University	Deng, Wu-Min	$ 815,283	$ 22,646.75	$ 792,636.25	6/12/2018	3/31/2021	No	No	No

NEWLY AWARDED GRANTS FISCAL YEAR 2017-2018

(Funding Year 2017-2018)

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

   Principal Investigator: John A. Copland, PhD

   Organization: Mayo Clinic Jacksonville

   Abstract of Proposed Research: This project was recently funded in April 2018. In this newly funded grant, the research team proposes to demonstrate antitumor synergy and survival benefit using the compound SSI-4, a novel potential therapy, in combination with anti-programmed cell death protein 1 (anti-PD1) and anti-programmed cell death ligand 1 (anti-PD-L1) antibody in triple negative and human epidermal growth factor receptor positive (HER2+) breast cancers as well as colon cancer and melanoma. Preliminary data shows that combined SSI-4, a novel inhibitor of fatty acid metabolism, with an immune checkpoint inhibitor cures 80% of mice using a HER2 positive mouse breast cancer. If successful, this will lead to clinical trials which may lead to long term survival for patients diagnosed with breast cancer, colon cancer and/or melanoma. The researchers also propose to study the mechanism by which SSI-4 sensitizes the tumor to the immune checkpoint inhibitor.

   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

   Abstract of Proposed Research: Tumor immunity requires antigen-specific cytotoxic T lymphocytes; however, to impact tumor growth these T cells must infiltrate into tumors, overcome a suppressive environment, and avoid becoming exhausted in the presence of persistent antigen. Conventional dendritic cells (DCs) are well established as the central inducers of the adaptive immune response, but emerging evidence suggests they may also play in supporting T cell activity within peripheral tissues, including tumors. In support of this, the researchers have published that TIM-3 (T-cell immunoglobulin and mucin domain containing-3) is highly expressed by tumor dendritic cells, and that TIM-3 blockade induces expression of the chemokine CXCL9 (C-X-C Motif Chemokine Ligand 9) in vitro and in vivo, thereby promoting T cell cytotoxic effector function. This project seeks to identify the DC activation pathways altered by TIM-3, determine if non-migratory DC maintain T cell function within tumors, and determine the role of CXCL9 expression by DCs in tumor immunity. These studies will delineate a putative DC regulatory pathway and improve understanding of the role of DCs within tumors, both factors that may have important implications for the design of combinatorial immunotherapies.

Presently, the researchers have found that using an in vitro system to screen for the activation of DCs during TIM-3 blockade, requires the presence of the STING (stimulator of interferon genes) gene (Tmem173). With this information, the researchers generated bone marrow chimeric animals lacking STING to determine if this pathway is required for the therapeutic response to TIM-3 blockade in combination with paclitaxel. Consistent with the in vitro observations, mice lacking STING within their bone marrow-derived cells were resistant to TIM-3 blockade. As the STING pathway is activated by the presence of DNA (deoxyribonucleic acid) within the cytoplasm of cells, these data suggested that TIM-3 was interfering with the ability of DCs to take up tumor cell DNA from the extracellular environment. The researchers therefore established a system to measure DNA uptake from tumor cells in vitro. Consistent with the hypothesis, TIM-3 blockade led to a significant increase in the ability of DCs to take up DNA. The data therefore indicates that TIM-3 is a negative regulator of DC activation through its ability to inhibit DNA uptake. This novel regulatory pathway has significant implications for cancer immunotherapy and autoimmunity. Results from these studies will help inform the design of clinical trials targeting TIM-3 in solid 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.

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

   Principal Investigator: Keiran Smalley, PhD

   Organization: H. Lee Moffitt Cancer Center

   Abstract of Proposed Research: Among all tumor types, melanoma has a high propensity to metastasize to the brain. Brain involvement is clinically evident in ~30% of melanoma patients (as high as 75% at autopsy) and the brain is often the major site of disease progression; even when extracranial disease is well controlled. Little is currently understood about the biology of melanoma brain metastasis and this represents a major knowledge gap that limits 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 (HDAC)-8 that reprogrammed melanoma cells to form brain metastases in animal models. Although both HDAC8-silenced and HDAC8 expressing melanoma cells were equally competent at forming lung and liver metastases, only the HDAC8 expressing cells established brain metastases. Melanoma cells that metastasize to the brain spend longer in the hostile environment of the circulation than those metastasizing to other organs; they also migrate along the blood vessels of the brain in a manner analogous to neural crest stem cells.

   The research team hypothesize that melanoma cells with high HDAC8 expression metastasize to the brain as a result of their increased resilience, enhanced interactions with the vasculature and their ability to evade immune detection. In this proposal, they will define the gene expression program that is controlled by HDAC8 and will determine how this cellular state permits the survival of melanoma cell in the circulation, enhances their migration into the brain and allows

them 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 (medications that can inhibit the effects of mutated proto-oncogene B-Raf (BRAF) proteins) and immunotherapies. This project is expected to bring important new insights into the biology of brain metastases allowing new therapies to be developed.

Follow On Funding: None at the time of reporting.

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.

Journals: None at the time of reporting.

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

   Abstract of Proposed Research: Solid tumors are unequivocally acidic due to elevated rates of glucose fermentation coupled with poor perfusion. Tumor microenvironmental acidity has been shown to promote local invasion, metastasis, and resistance to immune surveillance. It is axiomatic that adaptation to this acidic microenvironment is essential for tumor cells to survive and thrive and to out-compete the stroma into which they invade. In prior work, published in Cancer Research and Nature Communications, the research team has shown that acid adaptation is associated with chronic activation of autophagy and redistribution of the lysosomal proteins to the plasma membrane. These interrelated processes are key survival mechanisms adopted by tumor cells under acidic conditions. In subsequent studies, they have also observed that acid adaptation is accompanied by a robust and dramatic increase in the accumulation of cytoplasmic lipid droplets (“adiposomes”). The researchers hypothesize that adiposomes are coupled to autophagy and lysosomal redistribution and, hence, adiposome formation is a rapid readout for these other processes that together form an acid adaptation network. They are dynamic organelles that store neutral lipids surrounded by a shell of proteins (perilipins) and a phospholipid monolayer. Although a lipogenic phenotype is frequently observed in cancer, little is known about why they accumulate in acidic conditions or how acid signal perceived at the cell surface results in accumulation of lipid droplets. To identify if plasma membrane acid sensors are involved in transducing the signal, the research team used Clustered Regularly Interspaced Sort Palindromic Repeats (CRISPR)/CRISPR associated protein 9 (Cas9)-mediated depletion of major acid-sensing guanin nucleotide-binding protein (G-protein) coupled receptors in breast cancer: T cell Death-Associated Gene 8 (TDAG8) and Ovarian Cancer G protein coupled receptor 1 (OGR1). They observed that OGR1 (but not TDAG8) depletion inhibited acid-induced adiposome accumulation. In this project, the research team explores signaling downstream of OGR1 and functionally characterize OGR1 knockout cells to unravel the entire signaling cascade. They have also shown that acid-induced accumulation of lipid droplets persists even when cells were in de-lipidated serum, indicating de novo synthesis. Indeed, stable isotope 13C tracer studies indicate

that ketogenic amino acids resulting from autophagic protein degradation are the primary source of carbons for de novo synthesis in adiposomes. Paradoxically, fatty acid synthesis appears to occur contemporaneously with lipid β oxidation (βox), indicting a high turnover of adiposomes. Notably, inhibition of fatty acid synthesis or βox selectively killed cells under acidic, compared to neutral, conditions hence, this has identified a novel therapeutic vulnerability. The research team propose to further characterize the mechanisms controlling these metabolic pathways using gene editing in combination with 13C tracer metabolite analyses of fatty acid synthesis and degradation. Finally, they will assess if adiposomes are associated with acidosis and/or aggressiveness using mouse models and human tissue micro arrays. The researchers believe that the proposed studies will shed light on novel aspects of cancer biology and identify further new therapeutic opportunities.

Follow On Funding: None at the time of reporting.

Collaborations: The University of Florida, Department of Pathology, Immunology and Laboratory Medicine in Gainesville, FL has been involved in this research project through analytical measurement of lipid species using ultra-high pressure liquid chromatography (UHPLC) coupled to high resolution mass spectrometry. Currently, only Dr. Timothy Garrett (SECIM, University of Florida) has worked on this project as he is waiting on a post-doctoral student to start employment.

Journals: None at the time of reporting.

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

   Abstract of Proposed Research: The long-term objective of this project is to develop new cancer therapeutics for pancreatic cancer because it is the fourth-leading cause of cancer related death and has a shockingly-low five-year survival rate of <8%. The most common pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC). The research team hypothesize that PDAC tumors are driven to maintain high levels of intracellular polyamines by the genetic mutation in the Kras gene which occurs in the vast majority of PDAC cases. To explore this hypothesis, the researchers plan to develop new medicines (polyamine transport inhibitors, PTIs) which block the import of polyamines into cancer cells. These compounds when used with a polyamine biosynthesis inhibitor will starve the tumor of its polyamine growth factors and eventually shunt the tumor to programmed cell death (apoptosis). The project will also explore how polyamine transport genes are regulated and respond to external polyamine stimuli. They also will evaluate the new medicine in two mouse models of pancreatic cancer to show that this therapeutic approach works in vivo as well as on ex vivo human PDAC samples.

   Due to this grant being recently established at the University of Central Florida (UCF) in June 2018, the research team has only just begun their work. They have started the synthesis of the new PTIs and begun their evaluation of the PTIs that are owned by UCF. A success here will provide a new combination therapy for pancreatic cancers, a better understanding of how human polyamine transport is regulated and key preclinical data needed to assemble a future Investigational New Drug filing. They have also identified a new postdoc hire for the Molecular Biology post doc position and are currently interviewing additional candidates to fill the remaining

positions on their team.

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

   Principal Investigator: Barry I. Hudson, PhD

   Organization: University of Miami

   Abstract of Proposed Research: Metastasis is the major cause of death in women with breast cancer (BC), and despite extraordinary research efforts, effective therapies to treat metastasis are inadequate. Currently Dr. Hudson's lab has shown that the Receptor for Advanced Glycation End-products (RAGE) and its ligands represent a major mechanism driving BC to metastasize to other organs. Importantly, the research team has demonstrated that targeting RAGE with the small molecule inhibitor called FPS-ZM1 strongly inhibits BC metastasis in mice. Their preliminary data presented in this study, demonstrates that other RAGE inhibitors may be a more potent inhibitor of BC malignancy than FPS-ZM1. They hypothesize that therapeutic targeting of RAGE with orally bioavailable small molecule inhibitor TTP488 (a drug termed Azeliragon) will inhibit breast cancer cell invasion and metastasis. To test this hypothesis, the team will determine the anti-tumor efficacy of these RAGE inhibitors in a series of preclinical BC models, with the aim of generating preliminary data needed to further move RAGE inhibitors towards clinical trials and commercialization. The results of the current proposal will definitively establish the preclinical efficacy of RAGE small molecule inhibitors in impairing BC progression and metastasis.

   To date, the researchers have initiated studies by defining the biochemical and cellular effects of TTP488 on RAGE-ligand signaling in breast cancer (Aim 1). They have initiated the studies to test the role of RAGE inhibitors on both ligand binding and cellular function in the first part of Aim 1. These include Enzyme-Linked Immunosorbent Assay (ELISA) based assays, cell signaling assays, and oncogenic gene expression assays. Having optimized all the assays outlined in Aim 1, the research team will continue to test their central hypothesis by performing these assays.

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

   Principal Investigator: Ashok Saluja, PhD

   Organization: University of Miami

   Abstract of Proposed Research: Colon cancer is the second most common cause of cancer related deaths in United States. Every year over 50,000 people lose their lives to this disease. Most of these deaths are due to metastases. The liver is the most common site of metastases

from colon cancer. Eighty percent of colon cancer patients with metastases will have liver involvement and in half of these patient's their liver will be the only site of metastases. In fact, it is the presence and progression of liver metastases that primarily defines survival of these patients. Despite decades of progress in cancer, there are no good options for treating metastatic disease. Thus, a detailed understanding of the process of metastases is needed to change the course of disease in these unfortunate patients.

Why the liver is such a favorable site for development of metastases is unclear. As such the liver is considered an immune-privileged organ, which favors the induction of tolerance than induction of immunity. However, the reason why the liver is an immunotolerant organ and whether this immunotolerant phenotype contributes to the preponderance of metastases in the liver is not clear. The research team's preliminary results show that the depletion of gut microbiota prevents growth of liver metastasis and hint at the possibility that the inhibition of tumor growth observed with gut-microbiome depletion/alteration is likely to be an immune phenomenon. Based on this data the researchers have hypothesized that exposure to gut microbial antigens or their products causes immunotolerance and creates a permissive environment for the metastatic cancer cells to grow. This novel idea is evaluated in the current research project where the role of entero-hepatic link (circulation) in fostering liver metastasis by creating an immunotolerant environment in the liver will be tested. The goal is to find out whether the gut microbiome induces immunotolerance and promotes growth of liver metastases and if so, then elucidate the mechanism for this. An understanding of the mechanism and its key players will lead to development of novel strategies to treat liver metastases and even prevent progression of disseminated tumor cells into clinical metastases. This innovative study will provide potential therapeutic breakthrough in treating aggressive gastro-intestinal (GI) malignancies like colon cancer liver metastases by modulating entero-hepatic axis by routine antibiotics, probiotics or by targeting novel pathways identified in this research. These studies have the potential to change the outcome of patients with colon cancer liver metastases and thus have significant health impact.

Follow On Funding: None at the time of reporting.

Collaborations: This project is in collaboration with Dr. Prateek Sharma, Department of Surgery at the University of Miami; and two high school student volunteers: Anushka Gupta and Sachita Gupta.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

   Principal Investigator: Eric D. Wieder, PhD

   Organization: University of Miami

   Abstract of Proposed Research: In order to develop new treatments for cancer, and to better understand which cancer patients will benefit from a specific treatment, more sophisticated tools are continually being invented. These new technologies allow doctors and scientists to gain increasingly complex information on each person's cancer and will ultimately allow the customization of therapies to best benefit each patient and to provide the best possible outcomes. One such tool is the ability to look at tumor samples under the microscope and determine which cell types are in a tumor, what unique markers are on those cells and how other cell types within the tumor are interacting with it. This is done by taking a slice of tumor (biopsy), and staining it on

a slide and then taking a magnified picture of it using a microscope. There are various staining methods which allow pathology labs to identify various characteristics of tumors, but a more sophisticated way uses antibodies tagged with colors to be able to distinguish different markers on cells within the tumor. In most labs, it is typical to be able to look at one to four markers at the same time, although there is specialized equipment that can look at 10-12 at a time. A recently developed technology 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 50 markers or more. This technology was commercialized to look at single cells, but not tumor biopsies, within the last decade. More recently, this tool was modified to allow it to work to image cells in a tumor biopsy. Although there are over 60 installations of the recently developed single cell technology at academic and government research centers across the United States, and 30 installations of the new tumor imaging technology across the world, there are none for either single cells or tumors in all of Florida. This disruptive technology has begun to be used by scientists all over the world and results are beginning to be published. By purchasing this new equipment, it will be able to be used both for tissue samples and for cells in a suspension. The research team has numerous labs at the University of Miami and at Moffitt Cancer Center which have identified this technology as useful for their research. Creating this Imaging Center, which will be open to all cancer investigators in Florida, will greatly enhance Florida cancer researchers' ability to stay competitive in the developing areas of cancer research since soon, it will be required that these complex measurements will be included in any study that involves either heterogeneity of tumors (differences within them), or immune therapy. To date, the research team has ordered the equipment, renovated a laboratory to install the equipment, and identified the person to be trained to operate the equipment. The device is scheduled to be installed in August 2018, and training will commence and continue through the next reporting 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.

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

Principal Investigator: Shanta Dhar, PhD

Organization: University of Miami

Abstract of Proposed Research: During the past two months, the research team was focused on developing the materials needed for conducting in vitro and in vivo experiments. These materials included the multifunctional polymeric scaffold. They have followed the procedures already developed in their lab for production of the materials. They have further conducted extensive studies to understand the selective uptake of targeted nanoparticle in prostate specific membrane antigen (PSMA) expressing prostate cancer. These uptake studies indicated that the PSMA targeted nanoparticles accumulate in PSMA expressing LNCaP (lymph node carcinoma of the prostate) cells at a significantly higher concentration compared to the non-targeted nanoparticles indicating the selective targeting property of the engineered nanoparticle. Further, in PC3 (Human prostate adenocarcinoma) cells which do not express PSMA, the uptake of targeted nanoparticles was found to be similar to that of nontargeted nanoparticles further suggesting that the targeted nanoparticles can be specifically delivered to the PSMA expressing

prostate cancer. Thus, these nanoparticles will be able to deliver drug molecules specifically to prostate cancer more effectively avoiding other organs.

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 #8BC11:** A New Strategy to Increase Proteotoxic Cell Death in Prostate Cancer

    Principal Investigator: Carlos Perez-Stable, PhD

    Organization: South Florida Veterans Affairs Foundation

    Abstract of Proposed Research: Prostate cancer is a leading cause of death in men and when unresponsive to androgen deprivation therapy, it is known as castration-resistant prostate cancer (CRPC). Enzalutamide is a newly approved drug that inhibits androgen receptor activity and increases overall survival. However, most responding patients develop resistance to enzalutamide, indicating that new therapies are required to block CRPC. The research team has proposed a new strategy that increases proteotoxic stress with cyclophilin + proteasome inhibitors to promote apoptotic cell death in CRPC without toxic side effects. The team discovered a new chemotherapy strategy using an inhibitor of proteins in the cyclophilin family (CRV431) combined with inhibitors of the main protein degradation pathway, the proteasome (carfilzomib, ixazomib). Cyclophilins are required for proper folding of proteins so inhibitors of cyclophilins will increase misfolded proteins, which will further accumulate when combined with proteasome inhibitors to amplify proteotoxic stress and lead to cancer cell death. The preliminary data in CRPC and other cancer cells support the new CRV431 + carfilzomib combination chemotherapy strategy. Because the proteotoxic stress protective mechanism is already highly activated in cancer compared to normal cells, further increasing proteotoxic stress will have an irreversible lethal effect. Therefore, drug combinations that maximize proteotoxic stress may prove to be selectively toxic to cancer cells. To test their hypothesis, the team is identifying potential mediators that link ER stress/unfolded protein response to apoptotic cell death in CRV + carfilzomib or ixazomib that is treated with CRPC. The preliminary data collected, supports the idea that the new CRV431 + carfilzomib combination is more toxic to cancer cells including CRPC compared to non-cancer 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.

11. **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. To examine tumorigenesis, the researchers analyzed the conserved neoplastic tumor-suppressor genes (nTSGs) using the Drosophila wing imaginal disc model system, and found specific regions in which tumors always originate. Initially, experiments have been set up to explore how the modification of extracellular matrix (ECM) will affect tumorigenesis in the hotspot region.

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 B

FISCAL YEAR 2017-2018 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	$ 653,919.00	$ 817,399.00	3/17/2017	2/29/2020	Yes	No	No
7BC02	University of Florida	Judge, Andrew	$ 1,226,836	$ 545,260.00	$ 681,576.00	3/22/2017	2/29/2020	No	No	No
7BC03	University of Miami	Thomas, Emmanuel	$ 1,866,436	$ 497,716.00	$ 1,368,720.00	3/17/2017	2/28/2022	No	Yes	No
7BC04	H. Lee Moffitt Cancer Center	Gwede, Clement K.	$ 828,125	$ 220,832.00	$ 607,293.00	3/15/2017	2/29/2020	No	No	No
7BC05	H. Lee Moffitt Cancer Center	Smalley, Keiran	$ 1,468,200	$ 611,750.00	$ 856,450.00	4/12/2017	2/29/2020	No	No	No
7BC06	Florida Atlantic University	Wright, Amy E.	$ 622,683	$ 259,147.54	$ 363,535.46	3/27/2017	2/29/2020	No	No	No
7BC08	H. Lee Moffitt Cancer Center	Pilon-Thomas, Shari	$ 976,620	$ 434,048.00	$ 542,572.00	3/08/2017	2/29/2020	No	No	No

ACTIVE GRANTS FISCAL YEAR 2017-2018
(Funding Year 2016-2017)

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

   Principal Investigator: Anthony J. Capobianco, PhD

   Organization: University of Miami

   Progress Report: To effectively treat cancer in the future researchers need to develop therapeutics that are more specific and less toxic than the drugs available today. The research team identified a novel therapeutic target they called NACK. This protein is important in the Notch signaling pathway and is critical for many tumors to grow. This grant is to develop small molecule inhibitors to the protein that specifically inhibit he function of NACK in nanomolar (nM) concentrations, display minimal toxicity and have drug like characteristics. In this phase of the grant Dr. Capobianco's team is performing a structure activity relationship campaign designed to discover the most potent and specific compounds. Currently they have obtained potency of approximately 400-600 nM. Their goal is to identify a compound that is sub 100 nM. They are well on their way to achieving that goal. This part of the program is arduous as there are repetitive funds of chemical synthesis and screening to find the best compounds. In addition, they have been putting increasing efforts into understanding the mechanism of action in greater detail both biochemically and in cells. Furthermore, they are trying to co-crystalize the drug-protein complex to better aid their computer aided drug design. Along with their collaborator, Dr. Rhett Kovall, they have preliminary data suggesting they have identified conditions to crystalizes the protein and will soon be optimizing for co-crystals.

   Follow On Funding: None at the time of reporting.

   Collaborations: Rhett Kovall, PhD, Associate Professor of Molecular Genetics at the Department of Molecular Genetics, Biochemistry and Microbiology. University of Cincinnati, College of Medicine, Cincinnati, Ohio. Dr. Kovall's group (including a graduate student) is working on optimizing conditions for co-crystalization of NACK with select SAR compounds.

   Journals: None at the time of reporting.

   Patents: The invention entitled "Inhibitors of the Notch Transcriptional Activation Complex Kinase ("NACK") and Methods for Use" is provisionally patented by the University of Miami (UMIP-200 :MG Reference: 32286/51617 US).

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

   Principal Investigator: Andrew R. Judge, PhD

   Organization: University of Florida

   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, with or without the loss of fat. 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, the research team's focus in the past year has been on identifying such factors released from cancer cells and establishing their causality in cancer cachexia. In this regard, they have 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 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 they have spent the past year studying these proteins in this regard. The researcheres have found that treatment of skeletal muscle cells, or mice, with recombinant CXCL8 (r.CXCL8), r.human CXCL1 or r.mouse CXCL1 can induce significant muscle atrophy. In muscle cells, this is associated with acute activation of extracellular-signal-regulated kinase (ERK) 1/2, which has previously been implicated in cancer cachexia. They also grew human pancreatic cancer cells in vitro and 48 hours later collected the media containing factors which the cancer cells release, which is referred to as conditioned media (CM). When they treated muscle cells with this CM it induced significant atrophy of the cells. However, when they added a neutralizing antibody to CXCL8 or CXCL1 to the CM and treated muscle cells, the atrophy was significantly inhibited. Similarly, inhibition of the receptor CXCR2, also significantly inhibits atrophy of muscle cells in response to conditioned media from human pancreatic tumor cells. These findings demonstrate that IL-8 and CXCL1, which are released from various cancer cells, can induce muscle atrophy and are required for muscle cell atrophy induced by factors released from human pancreatic cancer cells. Since the CXCR2 receptor is a viable target to inhibit both IL-8 and CXCL1 signaling, they injected an adeno-associated viral (AAV) vector expressing a short hairpin-RNA (shRNA) targeting CXCR2 into the skeletal muscle of mice that were subsequently implanted with human pancreatic tumor cells. shRNA-CXCR2 injection significantly inhibited the increase in CXCR2 levels and completely blocked the tumor-induced skeletal muscle wasting. This suggests that signaling through CXCR2 is required for muscle wasting in response to human pancreatic tumors.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

   Organization: University of Miami

   Progress Report: Dr. Thomas and his team have collected clinical information and now have a comprehensive database for 1,974 patients with liver disease that are at increased risk of developing hepatocellular carcinoma (HCC). As described in Aim1 of the grant, they have started the cross-sectional analysis that will be carried out now in 1,974 patients to identify novel clinical covariates that may drive liver disease progression. Their goal is to identify covariates that may drive hepatocarcinogenesis in order to identify Floridians who are at risk earlier so that interventions can be employed. Emphasis in their future work will be focused on Floridians with

highest and intermediate risk of developing HCC and trying to generate a new risk calculator that incorporates Fibroscan. Toward their initial efforts to develop an liver cancer risk calculator that utilizes race/ethnicity, the research team has begun to develop new non-invasive prediction models for fibrosis and cirrhosis. Since cirrhosis is the most powerful predictor for the risk of developing HCC and because liver biopsies are being utilized less by the clinical community, the team believes these efforts will lay the foundation for their future work. Using multivariable statistical modeling, they are able to accurately predict cirrhosis (Metavir F4 fibrosis stage) utilizing noninvasive clinical markers. Importantly, they have submitted two follow on grants to the National Institutes of Health (NIH), in March and April 2018, and will receive notice of funding for these projects in December 2018. Furthermore, since starting this Bankhead-Coley Grant, the principal investigator (PI) has been awarded a 5-year, renewable grant from the NIH for $1.9 million. This NIH funded study is focused on understanding inflammatory 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 Dr. Thomas' team subsequently received a minority supplement to support a graduate student. Furthermore, they recently established the Florida HCV-HCC/Liver Cancer Consortium with Moffitt Cancer Center, University of Florida and Jacksonville Mayo Clinic through an initial meeting that was held at Moffitt Cancer Center in Tampa in October, 2017. The next meeting was held in Miami on May 7th, 2018 and the PI co-lead that meeting in Miami. It is anticipated that additional NIH grants will be submitted with work from this multi-institutional group that is focused on liver cancer and HCC.

Follow On Funding: None at the time of reporting.

Collaborations: This project formed the Florida HCV-HCC/Liver Cancer Consortium with Tampa Moffitt Cancer Center (Dr. Anna Giuliano-Center for Infectious Cancers), University of Florida Gainesville (Dr. David Nelson-Hepatology) and Jacksonville Mayo Clinic (Dr. Tushar Patel-Transplant Hepatology).

Journals: DeBose-Scarlett, A., Balise, R., Kwon, D., Vadaparmpil, S., Chen, S.X., Schiff, E.R., Ayala, G.P., Thomas, E.J., Obstacles to successful treatment of hepatitis C in uninsured patients from a minority population. (2018). Transl Med.16(1):178. doi: 10.1186/s12967-018-1555-y. PMID:29954391

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

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: The purpose of this study is to provide colorectal cancer prevention through early detection and regular continued screenings to improve the health outcomes of Floridians, more specifically focusing on Floridians who are being seen at Federally Qualified Health Centers (FQHC) which are vital medical safety-net homes for individuals who have limited access to health care.

   This project is conducted in partnership with two FQHC organizations located in Hillsborough and Pasco Counties. The project has two phases: Preparatory Phase I and Phase II, centered on conduct of a randomized controlled trial (RCT) in four clinic sites. The preparatory phase centered

on vital organizational and systems level activities that fostered increased support for colorectal cancer screening (initial and annual repeat). For example, systems-level activities involved electronic medical records (EMR) for assessing colorectal cancer screening, activation of the Community Advisory Board (CAB), and refinement of the Community CARES educational intervention materials to be used in the RCT.

In Phase 1, the preparatory phase, research activities have progressed extremely well, and the deliverables related to clinic readiness, intervention and data collection materials refinement, training of research coordinators, establishment of baseline Uniform Data System (UDS) rates, Electronic Medical Records (EMR) tracking of colorectal cancer screening, refinement of the intervention materials, and CAB engagement have been completed, with no impediments. In particular, the CAB members were instrumental in enhancing and reviewing the study instruments to ensure ease of administration, clarity of the materials and to minimize subject burden. Completion of Phase I activities culminated in initiation of the RCT (Phase 2 – current phase) In this second Phase, the research team aims, 1) to test whether C-CARES Plus (education + Fecal Immunochemical Test (FIT) + personalized components) compared with C-CARES (education + FIT) improves long-term FIT screening adherence among 328 individuals, 50-75 years of age, who are not up-to-date with CRC screening; and 2) to evaluate mechanisms through which C-CARES Plus may differentially improve initial CRC screening uptake and adherence. The research team started this phase on 3/29/2018 and 49 patients have been consented and enrolled in the reporting period. A total of four (4) patients had an abnormal (positive) FIT Kit results; these patients are being navigated to receive a follow up colonoscopy.

At this juncture, the direct benefit is to the enrolled study participants who received and used the FIT kit or other colorectal cancer screening. Once completed, this research is expected to explain how and why the intervention works, which patients benefit and which components to retain for effective adherence in a real-world setting. This study adds to the literature on test-specific impediments for Colorectal Cancer Screening (CRCS) and builds on strategies for increasing initial and repeat CRCS in FQHCs. If the Community CARES study is successful at also boosting FQHC-wide screening practices, a larger number of Floridians may benefit. Findings are readily translatable and inform the design of educational materials and interventions to increase CRCS in local FQHCs.

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

   Principal Investigator: Keiran Smalley, PhD

   Organization: H. Lee Moffitt Cancer Center

   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. At this time, there are no effective treatments for uveal melanoma and the majority of 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 drug called Mitogen-activated protein kinase (MEK) inhibitors.

Although these drugs are initially effective, patients typically only respond for a few months before relapsing. The central goal of the ongoing work is to develop combination drug strategies that increase the effectiveness of MEK inhibitors in uveal melanoma. In preliminary studies, they identified a class of enzymes called histone deacetylases (HDACs) that they found were critical in the development of resistance to MEK inhibitors. The research team consists of Dr Keiran Smalley (Moffitt Cancer Center, Tampa) an expert on melanoma biology and therapy, Dr. Jonathan Licht (University of Florida Cancer Center) an authority on epigenetics and 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 assay for transposase-accessible chromatin using sequencing (ATAC-Seq) and RNA-seq, and in particular how this landscape was regulated by three genes GNAQ, BAP1 and HDAC8. The second aim will focus upon how the three genes (e.g. GNAQ, BAP1 and HDAC8) regulate signaling networks in uveal melanoma cells and how these 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 first year of the grant the research team has made significant progress with all three aims. For Aim 1, Dr. Harbour's lab has generated multiple uveal melanocyte lines that express inducible mutants of GNAQ with concurrent BAP1 expression/silencing. They have performed some initial ATAC-Seq and RNA-Seq experiments and are now analyzing their large dataset. The Harbour lab has also been generating genetically defined mouse uveal melanoma cell lines that can be used for future animal studies. Dr. Licht's lab has been generating tools and reagents to perform a large-scale CRISPR screen to identify novel therapeutic vulnerabilities in the uveal melanoma cells. This work has included the introduction of CAS9 into the cells and the scaling up of the guide RNA libraries. His team is now poised to begin the screen that will generate further hits (and will identify novel therapeutic targets) for Drs. Harbour and Smalley to validate. Progress in Aim 2 includes proteomic mapping of the uveal melanoma cell lines by Dr. Smalley and a characterization of the changes in signaling that occur following MEK inhibitor treatment. These experiments have been analyzed and have demonstrated that MEK inhibition leads to increased signaling in AKT, YAP and ROR1. For Aim 3, the first series of animal studies have been completed demonstrating that use of a MEK inhibitor with an HDAC inhibitor is significantly better at treating uveal melanoma than either drug alone. At this time, studies are ongoing to determine whether the drug combination inhibits all of the targets identified from the proteomic experiments in Aim 2. Planning is now underway for a first in human clinical trial of the MEK+HDAC inhibitor combination in patients with uveal melanoma.

Follow On Funding: None at the time of reporting.

Collaborations: This project is a collaboration between the H. Lee Moffitt Cancer Center, The University of Florida and the University of Miami.

Journals: None at the time of reporting.

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

   Organization: Florida Atlantic University

Progress Report: Natural products or their derivatives represent over 48% of clinically approved cancer chemotherapeutics. The Harbor Branch Oceanographic Institution (HBOI) marine natural products chemical library represents a diverse source of genetically encoded small molecules that have actively co-evolved with cellular targets involved in both cell survival and death. The nodal protein survivin has been identified as an important target for intervention in a number of cancers including breast, colon and lung. Its expression correlates to poor prognosis and has a role in the aggressiveness of these diseases. It plays key roles as an anti-apoptotic protein; in mitosis; in drug resistance; in angiogenesis and in DNA repair response. A number of approaches to antagonize survivin's multiple functions have been explored but there remain very few small molecules that antagonize the activity of survivin. Discovery of additional antagonists will advance this field both in their understanding of basic biology of survivin and in clinical practice. This project aims to prove whether assaying chemically diverse natural products in the HBOI library will provide new lead molecules for therapeutic intervention against colon, lung and breast cancers.

To date, the research team has completed Aim 1 (optimization and implementation of the high content imaging (HCI) assay for survivin in A549 lung adenocarcinoma and DLD-1 colon carcinoma cancer cells). They have also completed 85% on research Aim 2. The results from testing the fractions and pure compounds in DLD-1 HCI assay, have identified a number of confirmed hits. Aim 3 is currently in progress and the team is working on six (6) lead organisms and have two pure active compounds for which structure elucidation is on-going. The research team has conducted nuclear magnetic resonance spectroscopy (NMR) and high performance liquid chromatography (HPLC) dereplication on the top hits and will continue as additional hits are confirmed in Aim 2. Hits have been identified in the HBOI Pure compound library and their activity confirmed in the HCI assays.

It is anticipated that the screening of the library will continue through the next quarter. Hits are being confirmed as they are identified and chemical dereplication is begun immediately after the hit is confirmed. Based on preliminary data, the researchers have found that >90% of hits in the primary screen have been confirmed when retested. Bioassay-guided fractionation has begun on the most potent hits that have strong potential for identifying novel inhibitors.

Follow On Funding: None at the time of reporting.

Collaborations: One doctoral student (Ms. Kirstie Tandberg) at Florida Atlantic University is currently conducting research for this project.

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

   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 are capable of recognizing and killing tumor cells. These T cells are ineffective due to suppressive factors in the cancer patient that allows tumors to "escape" from 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 re-activated 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. In order 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 of these 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 have been measured in initial experiments.

The mechanism that drives the expansion of MDSCs after non-myeloablative therapy has not been fully elucidated. Results from studies at the time of reporting suggest that Interleukin-6 (IL-6) may play a role in the expansion and/or function of MDSC after the induction of lymphopenia. In initial studies, loss of IL-6 improves tumor regression in melanoma-bearing mice treated with lymphodepleting chemotherapy and adoptive cell therapy with tumor-reactive T cells. Initial findings support a role for IL-6 in the suppressive capacity, not the expansion, of MDSC. Targeting MDSC can improve the anti-tumor activity of T cells in adoptive transfer protocols in melanoma bearing mice. As a consequence of these studies, novel approaches based on MDSC blockade and ACT may result and improve therapies for patients with melanoma and other advanced 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.

APPENDIX C

FISCAL YEAR 2017-2018 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
6BC02	University of Miami	Capobianco, Anthony	$ 1,343,732.02	$ 1,014,173.88	$ 329,558.14	4/20/2016	3/31/2019	No	No	No
6BC04	University of Florida	Tran, David D.	$ 1,784,753.25	$ 805,097.67	$ 979,655.58	3/04/2016	2/28/2021	Yes	No	Yes
6BC05	Mayo Clinic Jacksonville	Thompson, Aubrey	$ 1,064,624.44	$ 803,519.07	$ 261,105.37	4/12/2016	3/31/2019	Yes	Yes	No
6BC06	University of Miami	Antoni, Michael	$ 1,784,945.19	$ 813,160.94	$ 971,784.25	4/15/2016	3/31/2021	No	No	No
6BC07	University of Florida	Kladde, Michael	$ 681,887.44	$ 514,456.49	$ 167,430.95	3/31/2016	3/31/2019	Yes	Yes	Yes
6BC08	H. Lee Moffitt Cancer Center	Mahajan, Nupam	$ 1,329,860.86	$ 1,002,996.05	$ 326,864.81	4/15/2016	3/31/2019	No	Yes	Yes
6BC09	University of Florida	O'Dell, Walter	$ 1,445,736.61	$ 652,168.13	$ 793,568.48	3/19/2016	3/31/2021	Yes	Yes	No

ACTIVE GRANTS FISCAL YEAR 2017-2018
(Funding Year 2015-2016)

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

   Progress Report: During this reporting period, the research staff identified the compound NADI-351 (Notch inhibitor compound) 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 indicates 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, as evident by the decrease of Notch target genes expression Notch1, Hairy/Enhancer of Split genes (HES1), and has no effect on the housekeeping gene TATA-binding protein (TBP). 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 the mice experiments, as indicated by a villi and goblet cell architecture comparable to the one observed for the treatment with the vehicle of dimethyl sulfoxide (DMSO). Conversely, treatment with the gamma secretase inhibitor (DBZ, 30 µmol/Kg) leads to goblet cell metaplasia and a closer look of the intestinal crypts at higher magnification indicates disruption of the crypts using this concentration. These results indicate that NADI-351 does not induce goblet cell metaplasia, which is a marker of gastrointestinal toxicity.

   To obtain insight into the mode of action of NADI-351, staff treated OE33 cell line (esophageal adenocarcinoma) with either vehicle (DMSO or NADI-351) and performed 2XCSL DNA affinity pulldown as well as chromatin immunoprecipitation (ChIP) on HES1 promoter and tested the recruitment of individual Notch receptors (Notch1, Notch2 and Notch3). Noteworthy, esophageal adenocarcinoma cell lines and patient-derived xenograft models do not express Notch4, as previously shown in their reports. The data indicates that NADI-351 treatment leads to inhibition of Notch1 recruitment to the Notch ternary complex, chromatin on HES1 promoter, whereas no effect was observed on the recruitment of Notch2, Notch3 and Mastermind paralog 1 (Maml1) proteins. At the moment, the researchers are optimizing the conditions of the ChIP experiment to also determine the effect on other mastermind paralogs (Maml2 and Maml3). These preliminary data suggest NADI-351 selectively inhibits Notch1 recruitment to HES1 promoter, which has not been previously observed in other Notch inhibitors. Staff is still determining the effect of NADI-351 on Mastermind-like proteins. Since esophageal adenocarcinoma cell lines, such as OE33, do not express Notch4 protein, the team used the human breast adenocarcinoma cell line MDA-MB-231 (basal, triple negative breast cancer) that expresses all four Notch receptors Notch1-4 (Nagamatsu et al. (2014) Anticancer Res. 34: 69-80) 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 do not affect Notch4 recruitment to the complex. As expected, the gamma secretase inhibitor DAPT decreases recruitment of Notch1, Notch2 and Notch4 receptors to the Notch ternary 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.

Follow On Funding: None at the time of reporting.

Collaborations: Rhett Kovall, PhD, Associate Professor of Molecular Genetics at the Department of Molecular Genetics, Biochemistry and Microbiology. University of Cincinnati, College of Medicine, Cincinnati, Ohio. Dr. Kovall's group (including a graduate student) is working on optimizing conditions for co-chrystalization of proteins from the Notch ternary complex with the compounds.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

2. **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

   Progress Report: This grant supports the development of a novel treatment concept for patients with triple negative breast cancer (TNBC), a highly aggressive type of breast cancer with few effective treatments, in which quiescent, low-cycling disseminated tumor cells (DTCs) in patients with TNBC can be eliminated, and thus metastatic risks and death reduced, by first reactivating these quiescent DTCs to force them into fast cycling, thereby rendering them sensitive to conventional chemotherapy. If successful, this approach will have significant impact to Floridians afflicted with this cancer and most likely nationally as well. During the reporting period, research staff demonstrated in animal models of TNBC that pretreatment of these mice with a human-ready p38 inhibitor was able to significantly increase cycling of low-cycling DTCs, making them exquisitely sensitive to the chemotherapy drug Fluorouracil (5-FU), a widely-used cancer drug. A recent study in patients with TNBC showed that the drug capecitabine, an oral version of 5-FU, had activity against TNBC. TNBC mice treated with a sequential combination of the p38 inhibitor and 5-FU significantly prolonged survival with 40% of animals potentially cured, when compared to animals treated with 5-FU alone, in which no animal had long-term survival. Importantly the addition of the p38 inhibitor did not significantly increase toxicities caused by 5-FU. This work is protected by a patent listed below. With these findings, research staff are working with pharmaceutical companies to secure a supply of clinical grade p38 inhibitor to begin the planned trial in patients with TNBC.

   In addition, research staff have also focused on developing a suite of artificial intelligence (AI) algorithms, which create gene networks, predict master regulators of disease processes, and are also based on deep learning for image recognition. The original purpose was to develop a method to analyze the genomics data that will be generated in the planned clinical trial. Research staff has now created several proprietary technologies and discoveries protected by six (6) patent filings and one (1) technology disclosure. Several iterations of the algorithm have been generated with ongoing improvement testing. One of the early iterations achieved an accuracy rate of 99.6% based on the 60,000 hand-written digits dataset, which is competitive compared to similar state-of-the-art AI algorithms in the private sector. Once completed, this AI algorithm will be critical to further improvement of master regulator prediction for all human cancers. Data generated using this technology in brain cancer led to a $2.3 million business development grant from NIH/NCI

(See below). It can also be used for image recognition applications in radiological and histological datasets in diseases, and for mining large electronic medical records to identify clinical and disease trends and outcome predictions across geographic settings. In the next 2 years, research staff plans to develop a method to integrate genomics and gene network data, which is currently lacking, to understand complex biological processes such as cancer metastasis. For this grant, such an integration method is critical to understanding how mutations, either acquired in the primary tumor or in metastatic cells, contribute to the mechanism of low-cycling DTCs.

Follow On Funding: National Institute of Health - $2,300,000

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: The following patents have been published.

"A Molecular Cell Diary System." Provisional filed 2016. PCT filed February 2017: US/International. Patent Application Serial No. 62/301,813.

"Chemotherapeutic Resensitization Of Disseminated Cancer Stem Cells Through Reactivation by P38 Inhibition and IL-6 Inhibition -Chemotherapeutic Methods." Provisional filed in 2016. PCT filed February 2017: US/International Patent Application Serial No. 62/301,210.

"GeneRep and nSCORE: Method and Apparatus for Improved Determination of Node Influence in A Network." Provisional filed 2016. PCT filed October 2017: US/International Patent Application Serial No. 62/408,045.

"Core Master Regulators of Glioblastoma Stem Cells." U.S. Provisional Application filed November 2017: Serial No. 62/586,655.

"Direct Conversion of Brain Cancer Cells into Antigen-presenting Immune Cells." US Provisional Patent Application filed June 2017: Serial No. 62/522,887.

"Targeting Mechanisms of Immune Escape by Brain Cancer Cells to Synergize with Existing Cancer Immunotherapies." U.S. Provisional Patent Application filed June 2017: Serial No. 62/522,882.

"Reference Global Gene Networks and Master Regulators of 28 Major Human Cancers." 2017: UF#16847.

"Novel Factors for Cell Fate Conversions." 2017: UF#16818.

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

   Principal Investigator: Aubrey Thompson, PhD

   Organization: Mayo Clinic Jacksonville

   Progress Report: The research team carried out a comprehensive analysis of immune function gene expression in a very large (~1280) cohort of Human Epidermal Growth Factor Receptor-positive (HER2+) breast cancer samples from the N9831 trial of adjuvant trastuzumab in early stage HER2+ breast cancer. The staff designed a comprehensive panel of immune function genes based on Gene Ontology (GO) and expression data from The Cancer Genome Atlas (TCGA). This approach was used to quantify the abundance of 1252 immune function genes and to compare expression of these genes, individually and collectively, with therapeutic outcome.

Results from these analyses are currently being analyzed and conclusions are being, or are soon to be, validated using samples from the Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization (neoALTTO) and/or National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 trials.

One fundamental discovery from these initial experiments is that immune function gene expression tracks closely with favorable outcome following HER2-targeted therapy. This observation is a bit puzzling, since, in parallel studies, the researchers have shown that the total number of immune cells in a given tumor is not associated with outcome to a significant extent. Stated in another way, the research team determined that whereas total immune cells in a given tumor do not predict durable disease-free survival, it is nevertheless the case that genes expressed within those immune cells are associated with favorable outcome.

This seemingly paradoxical set of observations points to the complexity of the immune system, in which mounting an effective immune response to a tumor depends upon the number, types, and activity of immune cells. For this reason, it does not suffice to determine the total number of immune cells, one must identify, and ultimately quantify, those subsets of immune cells whose function within the tumor contributes directly to activation of an immune surveillance response. Identification and quantification comes down to the ability to quantify genes that can serve as surrogate markers for specific immune cell subsets. For example, the research staff have now defined a 17 gene “signature” that reads out the strength of the adaptive immune response in HER2+ tumors. This genomic “score” is associated with favorable outcome after HER2-targeted therapy, and, more importantly, identifies a subset of patients who receive additional benefit from combined therapy with anti-HER2 antibodies (trastuzumab) and small molecule receptor tyrosine kinase inhibitors (lapatinib) that block the enzymatic activity of the HER2 receptor. A patent application has been filed and a manuscript has been submitted describing this important step forward in reducing breast cancer mortality in Florida and elsewhere.

In parallel, the researchers have analyzed the relationship between age, immunological status, and outcome following HER2-targeted therapy. Remarkably, there was not an observable age-related decline in immune function in patients with HER2+ breast cancer who were treated with immunological therapy (trastuzumab). Neither did the team observe a significant increase in risk of relapse among young (versus old) patients. The studies identified a group of patients over 40 years of age with highly active immune systems who essentially never relapsed after surgery and adjuvant trastuzumab therapy. A manuscript is currently under review.

Follow On Funding: None at the time of reporting.

Collaborations: Worked with NSABP and NanoString. As well as the National Surgical Adjuvant Breast and Bowel Program, and the University of North Carolina.

Journals: Perez, E.A., Ballman, K.V., Mashadi-Hossein, A., Tenner, K.S., Kachergus, J.M., Norton, N., Necela, B.M., Carr, J.M., Ferree, S., Perou, C.M., Baehner, F., Cheang, M.C.U., Thompson, A. (2017). Intrinsic subtype and therapeutic response among HER2-positive breast tumors from the NCCTG (Alliance) N9831 trial. J. Nat’l Cancer Inst. 109(2): 1-8 doi:10.1093/jnci/djw207.

Patents: A patent entitled “Methods and Materials for Assessing and Treating Cancers” has been provisionally accepted. Application #: 62/679,431.

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

Principal Investigator: Michael H. Antoni, PhD

Organization: University of Miami

Progress Report: This study uses a novel technology to deliver stress management intervention groups to older distressed breast cancer (BCa) patients through a broadband connection to show for the first time the efficacy of stress management 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 research team 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 IV, and affective and immune/inflammatory processes in a randomized controlled trial using a remotely-delivered group Cognitive-Behavioral Stress Management (CBSM) intervention for older distressed women undergoing treatment for BCa. Over the past year, the study criteria has been revised to allow women in a wider disease stage range (now Stage 0 – III BCa) and age range (now > 50 yrs old) to enter the study; added a new breast cancer surgeon as co-investigator and broadened recruitment sites to include Jackson Memorial Hospital (JMH); conducted weekly reviews of patient census at breast cancer clinics at UMiami/Sylvester and its satellites and JMH; 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, the research team has accrued cases, executed the study protocol including conducting baseline assessments, randomizing cases and deploying the intervention conditions, collecting follow-up data, processing samples and conducting quality control monitoring of the data collection. All enrolled participants completed baseline assessments and were assigned to receive either the immediate stress management intervention condition or the Wait-List control (WLC). The on-line assessments have been successful. Participants are able to log-on using their computers, and complete the psychosocial assessment battery and the system is accurately transferring their responses to the lab’s 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. The intervention appears well received, the participants have been able to use the tablet-driven system with limited coaching, and any technical challenges have been 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 conferences.

Follow On Funding: None at the time of reporting.

Collaborations: Dr. Sara Czaja, Department of Psychiatry; Dr. Susan Kesmodel, Department of Surgery; Dr. Bonnie Blomberg and Dr. Daniella Frasca from the Department of Microbiology/Immunology; and Dr. Charles Carver and three graduate students in the Department of Psychology at the University of Miami.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

   Principal Investigator: Michael P. Kladde, PhD

   Organization: University of Florida

   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 (TSGs). 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 TSGs. The temporal order of changes in the locations of nucleosomes, histone modifications, and DNA methylation will be 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, assessed nucleosome positioning, using Assay for Transposase Accessible Chromatin sequencing (ATAC-seq). Results were compared to published datasets of changes in M series gene expression. Ninety-four target genes were identified with increased nucleosome density and reduced expression. Dr. Poudyal then performed Methylation Accessibility Protocol-individual templates (MAPit), an assay developed in Dr. Kladde's laboratory that detects positions of nucleosomes, transcription factors, and DNA methylation simultaneously. Collaborator, Dr. Nancy Nabilsi (Kappa Biosystems), processed the MAPit samples 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 will further characterize the aberrant epigenetic insults of the HRAS oncogene. The research team also conducted MAPit of 5 of 94 ATAC-seq target genes and found that all 5 accumulated increases in DNA methylation due to the presence of HRAS. These and other identified genes will be verified in human breast cancer specimens and test the hypothesis that changes in nucleosomes and histone modifications precede DNA methylation. For these latter studies, the HRAS oncoprotein will be introduced into pre-malignant M1 cells either on a virus or through direct editing of the HRAS locus, leveraging a collaboration to perform cutting-edge Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology. After oncogene introduction, gene expression and epigenetic features will be assayed over time to capture stepwise intermediates that accompany the transition from active to epigenetically silenced transcription. To do so, research staff have developed an improved and patentable MAPit-patch 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 could lead to the use of altered nucleosome occupancy as a novel, early biomarker of breast cancer.

   Follow On Funding: University of Florida Genetics Institute - $35,000; National Institute of Health - ≤ $6,837,684; Department of Defense - $1,392,993

Collaborations: Dr. Christopher Vulpe, Department of Physiological Sciences; Dr. Jonathan Licht and Daphné Dupéré-Richer from the Department of Medicine; Dr. Alberto Riva in the Interdisciplinary Center for Biotechnology Research; Dr. Nancy Nabilsi from Kappa Biosystems; and three undergraduate students in the Department of Biochemistry and Molecular Biology at the University of Florida.

Journals: Lorentsen, K.J., Cho, J.J., Luo, X., Zuniga, A.N., Urban, J.F. Jr., Zhou, L., Gharaibeh, R., Jobin, C., Kladde, M.P., and Avram, D., (2018) Bcl11b is essential for licensing Th2 differentiation during helminth infection and allergic asthma. Nat. Commun. 9:1679-1692. doi: 10.1038/s41467-018-04111-0 PMID: 29700302

Poudyal, R., Renne, R. and Kladde, M.P. (2017) Epigenetic regulation of gamma herpes viruses: A focus on Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8). In: Epigenetics of Infectious Diseases. Doerfler W. and Casadesús J., (eds.). Springer, New York, New York, pp. 15-46.

Patents: A patent entitled "Determination of Methylation State and Chromatin Structure of Target Genetic Loci" was filed in April 2018 by the University of Florida; international application PCT/US14/773,826.

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

   Principal Investigator: Nupam Mahajan, PhD

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: Prostate cancer is the third most common cause of cancer-related death in men in the United States. It is estimated that 161,360 men will be diagnosed and more than 26,700 men will die from the disease in this year. The majority of these deaths are caused by prostate cancer that becomes resistant to initial therapy and spreads to other sites, called metastatic castration-resistant prostate cancer. Undoubtedly, the foremost reason for transient effectiveness of the androgen deprivation therapy is a poor understanding of the molecular mechanisms driving progression to castration-resistant prostate cancer, which in turn has hampered development of new therapeutics. The research team demonstrated a newly discovered epigenetic mechanism which can lead to the development of castration-resistant prostate cancer. The team identified a novel drug (R)-9bMS that targets this epigenetic mechanism and may be able to combat the deadly form of the disease.

   Uncontrolled activity of male hormones, called androgens, contributes to the development of prostate cancer. One of the primary ways doctors treat prostate cancer is by inhibiting the activity of androgens by either surgically removing the testicles or with drugs that decrease androgen levels or activity. Unfortunately, even though most patients have early success with anti-androgen treatments, many patients eventually develop metastatic castration-resistant prostate cancer within two to three years. Castration-resistant prostate cancer is more difficult to treat and cure because scientists are unsure how it develops resistance to anti-androgen therapies.

   Dr. Mahajan's team performed an extensive set of experiments in prostate tumor cells and mice. The team discovered a protein, called activated Cdc42-associated kinase 1 (ACK1), also known as TNK2 that activates a pathway that causes the DNA-bound proteins called histones to undergo a type of modification called epigenetic modification. This modification was specifically accomplished by androgen receptor protein with the help of ACK1 around the region of the androgen receptor gene! This results in high levels and activity of the androgen receptor even

when prostate cancer cells have been treated with anti-androgen therapy. Following this discovery, the researchers developed a novel drug called (R)-9bMS that targets ACK1 and performed experiments to determine if it could block prostate cancer growth. They discovered that the ACK1 inhibitor blocked epigenetic modification of the androgen receptor gene and decreased its levels and activity. Importantly, the ACK1 inhibitor blocked the growth of prostate cancer cells that were resistant to the anti-androgen drug enzalutamide (also known as XTANDI) and decreased the growth of castration-resistant prostate tumors in mice. This discovery is highly relevant because almost two thirds of castration-resistant prostate cancer patients do not respond to enzalutamide. Overall, (R)-9bMS opens up as a new, and desperately needed, therapeutic option for those castration-resistant prostate cancer patients who either do not respond to enzalutamide or have acquired resistance, post-treatment.

Follow On Funding: National Institute of Health - $1,875,000

Collaborations: Mayo Clinic (a postdoctoral fellow and a research associate).

Journals: Mahajan, K., Malla, P., Lawrence, H., Chen, Z., Sinha, C.K., Malik, R., Shukla, S., Kim, J., Coppola, D., Lawrence, N. and Mahajan, N.P. (2017). ACK1 regulates histone H4 Tyr88-phosphorylation and AR gene expression in castration resistant prostate cancer. Cancer Cell, 31:790-803

Mahajan, N.P., Malla, P., Sharma, V., Sarnaik, A., Kim, J., Pilon-Thomas, S., Weber, J. and Mahajan, K. (2017). WEE1 epigenetically modulates 5-hmC levels by pY37-H2B dependent regulation of IDH2 gene expression. Oncotarget, 8(63):106352-106368

Mahajan, N.P., Coppola, D., Kim, J., Lawrence, H., Lawrence, N.J. and Mahajan, K. (2018). Blockade of ACK1/TNK2 to squelch the survival of prostate cancer stem-like cells. Scientific Report, 8(1):1954

Wu, X., Zahari, M.S., Renuse, S., Bharbuiya, M..A, Rojas, P.L., Stearns, V., Gabrielson, E., Malla, P., Sukumar, S., Mahajan, N.P. and Pandey, A. (2017). The non-receptor tyrosine kinase TNK2/ACK1 is a novel therapeutic target in triple negative breast cancer. Oncotarget, 8:2971-2983

Patents: None at the time of reporting.

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

   Principal Investigator: Walter O'Dell, PhD

   Organization: University of Florida

   Progress Report: This project is focused on better documenting and understanding the biological mechanisms and factors that influence radiation damage to the lung in breast cancer patients. Although radiation therapy (RT) is overall beneficial to breast cancer patients, around 14% will require medical intervention to treat lung toxicity following treatment. In particular, Dr. Walter and associates are interested in quantifying the reduction in lung toxicity afforded through the use of proton therapy versus standard x-ray based RT. The lab has developed tools to quantify both tissue and vascular damage from CT scans of the chest, and will correlate these changes with clinical findings of lung distress and patient survival, with pulmonary function (breathing) tests, blood markers of toxicity, and quality of life surveys. They plan to enroll 55 breast cancer patients to this study and acquire pre-treatment and follow-up scans at 6-month intervals for 2 years. The lab is, admittedly, behind in enrollment but enrollment has accelerated

in recent months. The researchers have acquired baseline and follow-up CT scans, blood draws, pulmonary function tests and surveys in 12 patients. The ultimate goal is to develop mathematical models of the biological process of lung tissue and lung blood vessel damage that includes the contributions of patient-specific factors such as age, gender and smoking history, and also considers the type of radiation treatment and the effects of chemotherapy. While awaiting collection of a sufficient number of patient data sets, the researchers have embarked on an exhaustive comparison of the 4 most common radiobiological models using imaging data from lung cancer patients. This comparison has been completed and the findings have been submitted for publication to the International Journal of Radiation Oncology, Biology, Physics. A PhD graduate student has now taken the conventional radiobiological models and formulated new extensions that will allow the research team to model the patient-specific and treatment factors.

Documenting the effects of radiation on blood vessels in the lung requires extraction of the vessel trees from the CT scans and accurate assessment of vessel branch size. Toward this objective, the team developed a method to mathematically model the appearance of a simulated vessel branch on a CT scan and use this to optimize the radius and trajectory of each branch in a vascular tree. A patent on this method was issued on Oct. 16, 2016. Dr. Walter and associates then endeavored to rigorously validate this method using three-dimensional print-out of a real lung arterial tree that was extracted from the chest CT scan of a human volunteer. Student researchers manually measured the radius of 69 branches in this 3D printed tree. They then scanned this tree in a clinical CT scanner and processed the images using the same software that was used on patient data sets. They verified that the method achieves accuracy of branch radii within 10% for branches as small as 2 mm in diameter. This work was published in the journal Medical Physics in September 2017.

Follow On Funding: None at the time of reporting.

Collaborations: Post-doctoral and undergraduate students in the Department of Biomedical Engineering and Medical Physics at the University of Florida (UF); Internal Medicine resident Dr. Dustin Begosh-Mayne; Karen Copeland in the UF Student Science Training Program; Samentha Etienne in the UF Cancer Research Training Opportunities for Outstanding Minority Leaders Program; and UF programmer Pooja Saini.

Journals: O'Dell, W.G, Gormaley, A.K., and Prida, D.A. (2017). Validation of the gatortail method for accurate sizing of pulmonary vessels from 3D medical images, Med Phys 44(12). Available on-line at Med Phys. 2017 Sep 14. doi: 10.1002/mp.12580.

Begosh-Mayne, D., Toffel, S., Siva Kumar, S., Okunieff, P., O'Dell, W. (2018). A comparison of dose-response characteristics of four NTCP models using a novel CT-based radiomic method to quantify radiation-induced lung density changes. Submitted to the International Journal of Radiation Oncology, Biology, Physics.

Patents: U.S. patent No. US 20140355858 A1 was issued on October 18, 2016. Medical Imaging Device that Locates and Sizes Blood Vessels and Airway Passages. Inventor: Walter O'Dell, PhD.

APPENDIX D

FISCAL YEAR 2017-2018 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
5BC04	University of Miami	Hu, Jennifer J.	$ 1,290,000	$ 1,171,750.00	$ 118,250.00	5/25/2015	11/15/2018	No	Yes	No
5BC07	H. Lee Moffitt Cancer Center	Haura, Eric	$ 1,686,887	$ 1,003,697.76	$ 683,189.24	5/25/2015	5/15/2020	No	Yes	Yes
5BC08	Sanford Burnham Medical Research Institute	Perera, Ranjan J.	$ 1,289,948	$ 1,182,452.33	$ 107,495.67	5/25/2015	11/15/2018	No	Yes	Yes

ACTIVE GRANTS FISCAL YEAR 2017-2018
(Funding Year 2014-2015)

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

Principal Investigator: Jennifer J. Hu, PhD

Organization: University of Miami

Progress Report: Breast cancer is the most frequently diagnosed cancer and the second leading cause of cancer death in American women; underserved minorities remain at a higher risk of dying from breast cancer in part due to a higher prevalence of a more aggressive breast cancer type, triple negative breast cancer (TNBC). Recent discoveries in genomics have improved breast cancer risk prediction and survival. However, translating this knowledge to precision medicine has not been possible due to the lack of prediction models of etiology and treatment response. Therefore, this research will bridge this critical scientific knowledge gap by developing novel prediction models of aggressive breast cancer, particularly TNBC. Dr. Hu and associates will test the working hypothesis that genetic variations, dietary factors, metabolite profiles, and tumor changes are associated with more aggressive TNBC and worse survival.

Aim 1: Genotyping and Triple Negative Breast Cancer (TNBC) Etiology - For Specific Aim 1, Dr. Hu's team proposed to use the newly developed Infinium OncoArray-500K BeadChip. During this funding period, the researchers have recruited new patients; genomic DNA has been isolated from whole blood ready for genotyping. They have completed 1,280 DNA isolation, quality and quantity checks, and genotyping.

Aim 2: Metabolomics in TNBC Etiology - For Specific Aim 2, Dr. Hu's team proposed to use frozen plasma samples for metabolomics assay. The researchers have completed the global metabolomics assays on a total of 6 batches of 48 samples: 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).

Aim 3: Next Generation Sequencing of Somatic Mutations in TNBC - For Specific Aim 3, Dr. H's team has proposed to use the Illumina TruSight RNA Pan-Cancer panel targeting 1,385 cancer-related transcripts and genes known to be involved in gene fusions, this approach enables analysis of cancer samples including formalin fixed paraffin embedded (FFPE) tissues and other limited samples. To date, Dr. Hu and associates have completed RNA isolation, quality/quantity check, and sequencing of 96 snap frozen biopsies.

Overall, the tea, has made significant progress on the project to date, particularly in patient enrollment (total N=1,500; 827 controls and 673 breast cancer cases), biopsy sample collection (total N=524), and laboratory assays. Rigorous data analysis effort and additional laboratory assays will take place during the No-Cost Extension funding period.

Follow On Funding: None at the time of reporting.

Collaborations: University of Florida Southeast Resource Center for Integrated Metabolomics (SECIM). A total of 2 MSPH students, a BS student, and a PhD student receive training and performing research under the research project.

Journals: Murphy, M.E., Liu, S., Yao, S., Huo, D., Liu, Q., Dolfi, S.C., Hirshfield, K.M., Hong, C.C., Hu, Q., Olshan, A.F., Ogundiran, T.O., Adebamowo, C., Domchek, S.M., Nathanson, K.L., Nemesure, B., Ambs, S., Blot, W.J., Feng, Y., John, E.M., Bernstein, L., Zheng, W., Hu, J.J., Ziegler, R.G., Nyante, S., Ingles, S.A., Press, M.F., Deming, S.L., Rodriguez-Gil, J.L., Haiman, C.A., Olopade, O.I., Lunetta, K.L., Palmer, J.R., Ambrosone, C.B. (2017). A functionally significant SNP in TP53 and breast cancer risk in African American women. NPJ Breast Cancer, 3(5). doi:10.1038/s41523-017-0007-9.

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Patents: None at the time of reporting.

2. **Grant #5BC07:** Signaling-Associated Protein Complexes for the Molecular Annotation of Therapeutic Vulnerabilities, Resistance-Associated Signaling & Tumor Heterogeneity in Lung Cancer

   Principal Investigator: Eric Haura, MD

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: The overarching goal of this Bankhead-Coley funded 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, data generated provide “proof-of-principle” that signaling complexes may represent an entirely novel class of predictive biomarker. 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. FDA-approved drugs exist 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 last year in a well-respected oncology journal (Clinical Cancer Research) and is currently being tested further as part of collaboration with Janssen Pharmaceuticals who recently opened a phase I trial at Moffitt and other locations to test a novel dual antibody against EGFR and MET. The Bankhead-Coley funding has been further leveraged to apply for and receive federal funding

to transition findings from the laboratory into clinical-grade diagnostic tests, work which is ongoing. Bankhead-Coley funding has enabled Moffitt 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 no concerns were raised in the most recent annual peer review. This includes: multiple national/international presentations, peer-reviewed publications and academic-industry partnerships.

Follow On Funding: National Institute of Health - $390,763

Collaborations: This project is in collaboration with the University of Colorado Medical School, Weill-Cornell Medical School, Albert Einstein Cancer Center, and the University Hospital Goettingen Germany.

Journals: Kim, E., Kim, J.Y., Smith, M.A., Haura, E.B., Anderson, A.R.A. (2018). Cell signaling heterogeneity is modulated by both cell intrinsic and extrinsic mechanisms: An integrated approach to understanding targeted therapy. PLoS Biology, 16(3):e2002930. (PMID: 29522507)

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Patents: None at the time of reporting.

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

   Principal Investigator: Ranjan J. Perera, PhD

   Organization: Sanford Burnham Medical Research Institute

   Progress Report: The research team made multiple contributions through the DOH 5BC08 grant to support State of Florida Cancer researchers. Some such contributions led to securing multiple grants, peer-reviewed publications, and high-level research collaborations. A few accomplishments are: (a) several NIH awards secured by Dr. Perera's collaborators; (b) planning a new genomics medicine symposium and a follow-up to the early 2017 symposium at the Institute; (c) several high-impact factor papers published; and (d) established a large number of research collaborations with State of Florida cancer researchers. The research team was also able to purchase and successfully install Next-seq 550 Illumina sequencing platform in the Genomics Core. This machine will allow State of Florida Cancer Researchers to process their sequencing samples at a much lower cost. Some investigators used the genomics core data to secure NIH grants, and awarded grants are listed in the progress report. One of the key highlights is the development of the Severe Combined Immune Deficiency (SCID) panel with collaborators at the University of South Florida. This panel will soon to be used in the clinical setting to test children with severe combined immune deficiency at All Children's Hospital in St. Petersburg

Florida. Members of the research team are actively involved in training and educating young scientists at Sanford Burnham Prebys and also at neighboring institutes and universities. The research team members presented their new assays and discoveries at national and international meetings, and some of their discoveries are highlighted in major scientific journals.

Follow On Funding: National Institute of Health - $466,538; Florida Breast Cancer Foundation - $199,937; the University of Florida Southeast Center for Integrated Metabolomics.

Collaborations: This project is in collaboration with the University of Central Florida, Burnett School of Biomedical Sciences, University of South Florida, the Florida Hospital/Translational Research Institute, Florida International University, Stanford University School of Medicine, and the Sanford Burnham Prebys Medical Discovery Institute.

Journals: Chen, Y., Yao, J., Eroshkin, A., Nerlakanti, N., Patel, A., Agarwal, N., Ngyuen, D., Li, J.L., Dhillon, J., Ma, Z., Shymalagovindarajan, S., Teer, J., Perera, R., Kim, Y., and Mahajan, K. (2017). The homeobox gene, HOXB13, regulates a mitotic protein-protein interaction network predictive of metastatic prostate cancer. Submitted 2017

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Patents: None at the time of reporting.

APPENDIX E

FISCAL YEAR 2017-2018 COMPLETED GRANTS,
Funding Fiscal Year 2016-2017

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
7BC07	University of Miami	Pei, Xin-Hai	$ 97,880	$ 97,880.00	$ 0.00	3/08/2017	2/28/2018	No	No	No
7BC09	H. Lee Moffitt Cancer Center	Tao, Jianguo	$ 97,880	$ 90,711.16	$ 7,168.84	3/14/2017	8/31/2017	No	Yes	No
7BC10	University of Miami	Wang, Gaofeng	$ 97,880	$ 97,880.00	$ 0.00	3/01/2017	8/31/2017	No	Yes	No
7BC11	University of South Florida	Tyson, Dinorah Martinez	$ 100,000	$ 80,000.00	$ 20,000.00	6/15/2017	6/30/2018	No	No	Yes

COMPLETED GRANTS FISCAL YEAR 2017-2018
(Funding Year 2016-2017)

1. **Grant #7BC07:** Targeting BRCA1 Deficient Breast Cancers

   Principal Investigator: Xin-Hai Pei, MD, PhD

   Organization: University of Miami

   Progress Report: Dr. Pei and associates discovered that heterozygous germline or epithelia-specific deletion of BRCA1 (gene) in p18- or p16-deficient mice activates platelet derived growth factor receptor beta (PDGFRβ) signaling, induces epithelial-to-mesenchymal transition (EMT), and leads to basal-like tumors. BRCA1 binds to PDGFRβ locus and represses its transcription. Targeted deletion of PDGFRβ in Brca1-deficient tumor cells promotes apoptosis, induces mesenchymal-to-epithelial transition (MET), and suppresses tumorigenesis. Pharmaceutical inhibition of PDGFRβ and its downstream target protein kinase C alpha (PKCα) suppresses Brca1-deficient tumor initiation and progression. Inhibition of PDGFRβ or PKCα activity selectively kills BRCA1-deficient cancer cells. Expression of BRCA1 is inversely related with that of PDGFRβ and PKCα in breast cancer samples. Together, the work offers the first genetic and biochemical evidence that PDGFRβ-PKCα signaling is repressed by BRCA1, which establishes PDGFRβ-PKCα signaling as a therapeutic target for BRCA1-deficient breast cancers.

   Follow On Funding: None at the time of reporting.

   Collaborations: This project was in collaboration with Mr. Cheng Fan, a Bioinformatics Specialist at the University of North Carolina at Chapel Hill; Dr. Anthony Capobianco, a professor at the University of Miami; and Dr. Tan Ince, a professor and pathologist at the University of Miami.

   Journals: None at the time of reporting.

   Patents: None at the time of reporting.

2. **Grant #7BC09:** Ibrutinib Resistance Mechanism in Mantle Cell Lymphoma (MCL)

   Principal Investigator: Jianguo Tao, MD, PhD

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: This study revealed that Myc, a proto-oncogene protein, functions as a key driver for mantle cell lymphoma (MCL) aggressive progression and tumor microenvironment (TME)-mediated drug resistance, the phenotype observed in ibrutinib resistance (IR) MCL, 2) Increased Myc expression was detected in IR MCL cell lines and patient samples, 3) significant overlap of Myc-induced and IR associated kinome (kinase) alterations and 4) silencing Myc with bromodomain and extra terminal domain (BET) inhibitor JQ1, down-regulates Myc expression, produces sustained growth inhibition in IR cells, overcomes IR and synergizes with ibrutinib to suppress MCL growth and survival in IR MCL cells. Thus, the research team tested and demonstrated the hypothesis: Myc-mediated kinome reprogramming promotes MCL IR and is regulated by BET bromodomain proteins.

   The recent data supports that adaptive responses leading to IR involve reprogramming of the kinome through reactivation of transcriptional upregulation and induction of multiple kinases/pathways, and thus, targeting one kinase or pathway will not be as sufficient as targeting

kinase remodeling as a whole. The team's pathway analysis identified PI3K-AKT-mTOR (an intracellular signaling pathway important in regulating the cell cycle) signaling pathway as a convergent node of downstream kinase signals for IR, resulting in enforced TME-lymphoma interactions, promoting MCL growth and drug resistance. Thus, targeting phosphatidylinositol-3-kinase and mammalian target of rapamycin (PI3K-AKTmTOR) is significantly effective but may not be sufficient to completely eliminate MCL, 3) targeting the key upstream regulator, Myc/bromodomain containing 4 (BRD4), of kinome reprogramming suppressed activation of majority of kinases having potential role in IR, thus providing rational for combination therapy of Myc inhibitor with inhibitors of PI3K-AKTmTOR for durable efficacy. To this end, the researchers approached this problem with the hypotheses that co-targeting Myc and PI3K-AKT-mTOR pathway has a synergistic and more durable anti-MCL activity against IR. Thus, they used multiple experimental conditions and both parental and IR MCL cell lines and revealed that combinatorically targeting of Myc and PI3K-AKT-mTOR1 synergistically and cooperatively overcome drug resistance.

Most recently, they observed sustained activation of RNA polymerase II supporting that transcriptional reprogramming plays a critical role in ibrutinib resistance development, and are potential target for bromodomain-containing protein (BRD4) inhibition to overcome the drug resistance.

Follow On Funding: None at the time of reporting.

Collaborations: Project is in collaboration with Jun Qi, PhD, Assistant Professor, Department of Cancer Biology, Dana Faber Cancer Institute, Harvard Medical School.

Journals: Zhao, X., Lwin, T., Silva, A., Shah, B., Tao, J., Fang, B., Zhang, L., Fu, K., Bi, C., Li, J., Jiang, H., Meads, M.B., Jacobson, T., Silva, M., DIstler, A., Darville, L., Zhang, L., Han, Y., Rebatchouk, D., Di Liberto, M., Moscinski, L.C., Koomen, J.M., Dalton, W.S., Shain, K.H., Wang, M., Sotomayor, E. & Tao, J. (2017). Unification of de novo and acquired ibrutinib resistance in mantle cell lymphoma. Nature Communications, vol. 8, 14920. doi: 10.1038/ncomms14920.

Jiang, H. , Lwin, T. , Zhao, X. , Ren, Y. , Li, G. , Moscinski, L. , Shah, B. and Tao, J. (2018). Venetoclax as a single agent and in combination with PI3K-MTOR1/2 kinase inhibitors against ibrutinib sensitive and resistant mantle cell lymphoma. Br J Haematol. doi:10.1111/bjh.15079.

Patents: None at the time of reporting.

3. **Grant #7BC10:** Epigenetic Prevention of Breast Cancer Progression by Vitamin C

   Principal Investigator: Gaofeng Wang, PhD

   Organization: University of Miami

   Progress Report: Genetic and epigenetic variations underlie differences in cancer drug response. Unlike genetic variation, epigenetic variation is reversible and therefore provides an opportunity to improve drug response by targeting epigenetic regulators. Bromodomain and extra-terminal (BET) proteins, in particular bromodomain containing 4 (BRD4), play key roles in cancer by binding to acetylated histones to promote the expression of oncogenes. BET inhibitors (BETi) are a group of compounds that block BET proteins from binding to the acetylated lysines in histones. Preclinical studies have shown that BETi are effective in treating breast cancer, especially triple negative breast cancer (TNBC), which affects many Floridian women. However, severe side effects of BETi at effective doses are significant as shown in clinical trials and are a

limitation to the clinical implementation of BETi in patient care. The preliminary data from study suggest that oral vitamin C, which can be delivered by citrus and other vitamin C rich diets, increased an epigenetic mark termed 5-hydroxymethylcytosine (5hmC) in breast cancer cells toward levels observed in normal epithelial cells, altered the transcriptome, and induced apoptosis mainly by an increased expression of TNF-related apoptosis-inducing ligand (TRAIL). High-throughput screening further revealed that vitamin C improves the response of breast cancer cells to a number of structurally unrelated BETi including JQ1, I-BET762, I-BET151, and CPI-203. Vitamin C enhances the efficacy of BETi by decreasing the acetylation of histone H3 and H4 mainly via upregulation of histone deacetylase 6 (HDAC6). Addition of vitamin C caused a synergistic inhibition of the binding of BRD4 to acetylated histones, mediating the improved BETi efficacy. Preliminary results also indicated that in vitro, treatment with JQ1 together with vitamin C, especially at 100 µM which is achievable in vivo by diet and dietary supplements, synergistically induced apoptosis and blocked the invasion of breast cancer cells. In vivo, dietary vitamin C supplementation enhanced the inhibitory effect of JQ1 on breast cancer tumor growth in mice. BETi are promising cancer drugs that can effectively treat breast cancer, but also cause severe side effects in clinical trials. Further studies are warranted to study if vitamin C can improve the efficacy and reduce the toxicity of BETi therapy in model systems and eventually in breast cancer patients.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Sant, D.W., Mustafi, S., Gustafson, C.B., Chen, J., Slingerland, J.M., Wang, G. (2018). Vitamin C promotes apoptosis in breast cancer cells by increasing TRAIL expression. Scientific Reports. 8:5306. doi:10.1038/s41598-018-23714-7.

Patents: None at the time of reporting.

4. **Grant #7BC11:** Women at Work: Multi-Ethnic Comparison of Cancer Survivors with Low-Status Jobs

   Principal Investigator: Dinorah Martinez Tyson, PhD

   Organization: University of South Florida

   Progress Report: There is scant knowledge about work outcomes among women cancer survivors who were employed in low-status occupations at time of diagnosis. Work outcomes have substantial implications for their short- and long-term economic, physical and psychosocial well-being. To understand the underlying work environments that exacerbate health disparities and affect cancer morbidity, particularly among minority cancer survivors, this funded project explored work-related decisions and challenges of cancer survivors in low-status occupations and developed a survivor and employer advisory board.

   To date, the research team has successfully completed their data collection. A total of 33 survivors participated in the in-depth interviews and questionnaire administration. The interviews have been transcribed and they are currently analyzing the data through Atlas 71Preliminary data analysis conducted on a sample of interview transcripts show three emerging themes as relevant to the work experience of women cancer survivors: (1) the impact of physical and mental side effects of cancer treatment on the ability to work, work arrangements and confidence in the ability to carry out tasks, (2) social support as a mean to cope with cancer experience and its positive spillovers on employment; (3) the ambivalent role of disclosure as both a facilitating and hindering

factor of employment. All participants disclosed their cancer diagnosis to their supervisors. Majority of participants felt comfortable to disclose their cancer diagnosis to supervisor and colleagues, did not regret their decision and felt they received support. Timing of disclosure seems to be different across groups considered. While Caucasian and Hispanic women disclosed their status immediately after diagnosis, African American women in the sample chose not to disclose their cancer diagnosis immediately to their supervisor. In comparing women who stopped working and continued to work, women who stopped working reported that their health took priority over work and did not communicate with their supervisor immediately after diagnosis. In addition, they have developed a committed and knowledgeable advisory board whose input has been invaluable and who advised on two grant proposals that build on this work (one internal and one external).

Follow On Funding: University of South Florida College of Public Health - $23,980

Collaborations: This community-based participatory research project is in partnership with both local West Central Florida and national cancer survivorship organizations. The research team has also developed a community advisory board that comprises of survivors, employers, and community and national cancer survivor advocates. The final community advisory board includes 10 survivors, employers, and community and national cancer survivor advocates. There are also two doctoral and two undergraduate students from the University of South Florida working on this project.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

APPENDIX F

FISCAL YEAR 2017-2018 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
5BC02	Mayo Clinic Jacksonville	Radisky, Derek C.	$ 1,200,953	$ 1,100,873.58	$ 100,079.42	5/24/2015	5/15/2018	No	Yes	Yes
5BC03	H. Lee Moffitt Cancer Center	Kim, Minjung	$ 970,758	$ 889,861.50	$ 80,896.50	5/19/2015	5/15/2018	No	Yes	No

COMPLETED GRANTS FISCAL YEAR 2017-2018
(Funding Year 2014-2015)

1. **Grant #5BC02:** Development of Assays for Individualized Breast Cancer Risk Prediction

   Principal Investigator: Derek C. Radisky, PhD

   Organization: Mayo Clinic Jacksonville

   Progress Report: The overall objective of this project was to develop clinical assays for individualized breast cancer risk prediction that will allow for improved early detection and targeted cancer prevention. It has been estimated that existing breast cancer prevention agents could reduce disease incidence by three fourths, but these agents are rarely used because of a lack of knowledge about which women are most at risk for developing breast cancer. Over one million women per year in the US undergo breast biopsies with benign findings, resulting in a diagnosis of benign breast disease (BBD), and yet beyond this catch-all diagnosis, biopsy findings are not currently used to inform cancer risk models or clinical decisions. The research team has previously shown that these breast tissue biopsies contain critical information that predict later cancer susceptibility. Their approach in this research grant was to fill the critical gap in individualized risk prediction and develop NanoString-based clinical assays that use RNA derived from BBD tissue biopsies. Here, the researchers used patient cohorts consisting of women who were diagnosed with BBD and who subsequently developed breast cancer to identify potential cancer risk biomarkers, and then developed NanoString-based assays based on these markers. Dr. Radisky and associates used the Mayo Clinic BBD cohort, which consists of ~14,000 primarily Caucasian women to identify two test sets: 1) women who subsequently developed estrogen receptor positive (ER+) breast cancer, ER- breast cancer, or did not develop cancer, and 2) women who subsequently developed poor prognosis breast cancer vs. did not develop breast cancer. They defined their patient sets, isolated RNA, discovered potential cancer risk biomarkers using RNA-Seq, and defined NanoString-based assays for validation and model development. While pursuing this project, a collaboration was formed with Dr. Michele Cote at Wayne State University in Detroit, through which the research team expanded their investigation to include the Detroit BBD cohort, which consists of ~4,000 African American women, of which 222 breast cancers have occurred to date. Completion of the ER+/ER-/control project has led to a two-stage test: first, in which they classify patients as at cancer risk or not, and then second, to classify whether at risk are more likely to develop ER+ or ER- cancer. For the poor prognosis project, the researchers have generated a NanoString-based assay, testing of which is now underway. For the collaborative project with Wayne State University, they identified a cohort of African-American women with BBD who went on to develop breast cancer, identified potential risk biomarkers, and developed and tested a NanoString-based assay for risk prediction in this underserved population. In addition to these ongoing efforts, the work performed in this project has led to identification and characterization of highly valuable patient cohorts and also resulted in the development of optimized methodology for isolation of usable RNA from decades-old archival patient samples that have been used in a number of published manuscripts and that have provided critical background and preliminary data for one funded R01 grant, two submitted R01 applications, and one submitted DOD BCRP application.

   Follow On Funding: National Cancer Institute - $640,367

   Collaborations: This research project has led to several critical and important new

collaborations, including work with Dr. Michele Cote at Wayne State University in Detroit; and Dr. Melissa Troester at the University of North Carolina.

Journals: Degnim, A.C., Winham, S.J., Frank, R.D., Pankratz, V.S., Dupont, W.D., Vierkant, R.A., Frost, M.H., Hoskin, T.L., Vachon, C.M., Ghosh, K., Hieken, T.J., Carter, J.M., Denison, L.A., Broderick, B., Hartmann, L.C., Visscher, D.W., Radisky, D.C. (2018). Model for Predicting Breast Cancer Risk in Women With Atypical Hyperplasia. J Clin Oncol. 20:JCO2017759480. doi: 10.1200/JCO.2017.75.9480. [Epub ahead of print] PMID: 29676945.

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Santen, R.J., Radisky, D.C., Degnim, A., Frost, M.H., Vachon, C.M., Ghosh, K., Guestini, F., McNamara, K.M., Sasano, H. (2017). Aromatase expression in atypical ductal hyperplasia in women. Breast Cancer Res Treat. Mar 23. doi: 10.1007/s10549-017-4184-x. [Epub ahead of print] PMID: 28337664.

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Denison, L.A., Carter, J.M., Hartmann, L.C., Visscher, D.W. (2016). Extent of atypical hyperplasia stratifies breast cancer risk in 2 independent cohorts of women. Cancer.Jun 28. doi: 10.1002/cncr.30153. [Epub ahead of print] PMID: 27352219. PMCID: PMC5030128.

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Patents: None at the time of reporting.

2. **Grant #5BC03:** Elucidating the Role of R-Ras Activation in Melanoma Tumorigenesis

   Principal Investigator: Minjung Kim, PhD

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: Melanoma is the deadliest form of skin cancer. Abnormally activated Ras proteins have been reported to contribute to melanoma formation. The Ras family includes many closely related, but unique, forms of Ras proteins such as H-, K-, N-, R-, and M-Ras, and mutations that activate N-Ras have been observed in 15~20% of melanoma patients. In addition, Ras proteins can be activated through the inactivation of Ras guanosine triphosphatase (GTPase)-activating proteins (RasGAPs). Previously, Dr. Kim and associates have shown that RASA1, one of the RasGAPs, is inactivated in melanoma by inactivating mutations or by loss of protein. RASA1 suppresses melanoma growth by inhibiting R-Ras and RalA (downstream target of R-Ras) activity and confers decreased response to BRAF (a gene) targeted therapies. They also observed that melanoma patients with activating BRAF mutations (the most common mutations occurring in 40~60% of melanoma patients) survived longer when they express RASA1 at high levels compared to patients expressing low levels of RASA1, suggesting importance of RASA1/R-Ras pathway in BRAF mutant melanomas.

   This study endeavored to understand the role of R-Ras activation in melanoma tumorigenesis, they proposed to Elucidate aberrations in R-Ras signaling pathway in melanoma (Aim 1), to test the cooperation of R-Ras and B-Raf activation in melanoma tumorigenesis (Aim 2), to test the value of R-Ras as a therapeutic target (Aim 3), and to assess the biological impact of RASA1 inactivation and subsequent R-Ras activation on initiation and progression of BRFV600E-driven melanoma (Aim 4). During the period from July 1, 2017 to May 15, 2018, the researchers observed that R-Ras is activated in a majority of human melanoma specimens harboring BRAF

V600E/K mutation and reduced R-Ras expression suppressed tumor growth and inhibited RalA activation. Colony growth in 3D (measurement of tumor forming potential) was significantly inhibited by BRAF inhibitor (vemurafenib), Ral inhibitor (BQU57), or R-Ras suppression, which was further suppressed by combined inhibition of BRAF with Ral or R-Ras. This supports the co-targeting strategy of BRAF and R-Ras/Ral-A pathways. In addition, loss of Rasa1 expression cooperated with BRAF activation leading to earlier onset of melanoma formation and emergence of metastases in a genetically engineered mouse model. Melanomas developed in Rasa1 mutant mice showed R-Ras activation. All these data support that RASA1/R-Ras/RalA pathway drives melanoma formation in collaboration with BRAF activation and suppression of the R-Ras pathway can be a novel therapeutic target.

Follow On Funding: None at the time of reporting.

Collaborations: This project is in collaboration with Dr. Michael Mann at Moffitt Cancer Center, and research intern Elliott Chon (an undergraduate student at the University of South Florida).

Journals: Sung, H., Kanchi, K.L., Messina, J., Lee, J.H., Kim, Y., Dees, N., Ding, L., Teer, J., Yang, S., Sarnaik, A., Sondak, V.K., Mulé, J.J., Wilson, R.K., Weber, J.S., and Kim, M. (2016). Inactivation of RASA1 promotes melanoma tumorigenesis via R-Ras activation. Oncotarget, 7(17): 23885-96. doi:10.18632/oncotarget.8127.

Patents: None at the time of reporting.

James and Esther King Biomedical Research Program

APPENDIX G

FISCAL YEAR 2017-2018 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
8JK01	Florida Atlantic University	Fields, Gregg	$ 708,044	$ 76,545.00	$ 631,499.00	5/11/2018	4/30/2021	No	No	No
8JK02	H. Lee Moffitt Cancer Center	Permuth, Jennifer	$ 1,360,857	$ 113,404.00	$ 1,247,453.00	5/03/2018	3/31/2021	No	No	No
8JK03	H. Lee Moffitt Cancer Center	Kumar, Nagi	$ 708,044	$ 35,402.00	$ 672,642.00	5/03/2018	3/31/2023	No	No	No
8JK04	University of Florida	Kaye, Frederic J.	$ 1,360,857	$ 22,681.00	$ 1,338,176.00	6/06/2018	3/31/2023	No	No	No
8JK05	University of Florida	Kusmartsev, Sergei	$ 816,514	$ 68,043.00	$ 748,471.00	5/04/2018	3/31/2021	No	No	No
8JK06	University of Florida	Hayward, Linda F.	$ 816,514	$ 68,043.00	$ 748,471.00	5/07/2018	3/31/2021	No	No	No
8JK07	University of Miami	Saluja, Ashok	$ 816,514	$ 68,043.00	$ 748,471.00	4/25/2018	3/31/2021	No	Yes	No
8JK08	University of Miami	Merchant, Nipun	$ 99,999	$ 49,999.00	$ 50,00.00	4/25/2018	9/30/2018	No	Yes	No
8JK09	University of South Florida	Ghansah, Tomar	$ 816,514	$ 45,371.00	$ 771,143.00	4/17/2018	3/31/2021	Yes	No	No

NEWLY AWARDED GRANTS FISCAL YEAR 2017-2018

(Funding Year 2017-2018)

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

   Principal Investigator: Gregg B. Fields, PhD

   Organization: Florida Atlantic University

   Abstract of Proposed Research: Matrix metalloproteinase 14 (MMP-14)/membrane type 1 metalloprotease (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 a number of tobacco-related cancers and the collagen-cleaving ability of MT1-MMP is critical to the progression of a number of tobacco-related cancers. A mechanistic examination of MT1-MMP at the cell surface would unravel the influences of cell surface binding partners on MT1-MMP activities and set the stage for the development of unique MT1-MMP inhibitors. This grant utilizes cutting-edge technologies to examine, on a molecular level, how a cell surface protease (MT1-MMP) functions in its native environment. In addition, the cell surface nature of MT1-MMP will be utilized to design novel inhibitors. The specific aims to achieve these goals are as follows: (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.

   The project has just started. In the initial months, progress has been made in three areas. First, staff have begun the expressional of four enzymes, full-length MT1-MMP, 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 oligomer (PICCO) library. Second, they have obtained quantitative results for the cell-based assay using wild-type, full-length MT1-MMP and several mutants. The researchers have 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 successfully evaluated in the three-dimensional assay. Third, researchers have compared the production of MT1-MMP in three-dimensional melanoma spheroid models with the same cells grown under standard two-dimensional conditions. It as found that MT1-MMP production is greatly enhanced in three-dimensional invading spheroids embedded in a type I collagen matrix. The three-dimensional system closely mimics the environment that melanoma encounters during the metastatic (cancer spreading) 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.

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

   Principal Investigator: Jennifer B. Permuth, PhD

   Organization: H. Lee Moffitt Cancer Center

   Abstract of Proposed Research: The goals for this project are to create a comprehensive state-wide biobank to conduct pancreatic cancer (PC) research that will impact several racial/ethnic groups. The secondary goal will be to utilize the biobank to evaluate for the first-time cachexia in the context of PC racial disparities. The specific aims for this study are 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.

   The research team has updated the study protocol to reflect enhancements in methodology, drafted an informed consent document and formulated a baseline study questionnaire. The team is also finalizing core facility and laboratory operating procedures for collaboration and have started to assemble the community advisory panel. The future plans are to submit study-related documents and obtain scientific and regulatory approval at the coordinating sites and all participating sites, then begin the process of training staff at these participating sites.

   Follow On Funding: None at the time of reporting.

   Collaborations: None at the time of reporting.

   Journals: None at the time of reporting.

   Patents: None at the time of reporting.

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

   Principal Investigator: Nagi B. Kumar, PhD

   Organization: H. Lee Moffitt Cancer Center

   Abstract of Proposed Research: 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 has shown that curcumin (CUR) and omega 3 fatty acids (ω-3 FA) are effective at suppressing two signaling pathways [Signal transducer and activator of transcription molecules phosphorylated (Stat3P) and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB)] - that are relevant to lung carcinogenesis- resulting in suppression of proliferation of human lung tumor lines and inflammation responses. More recently, strong evidence has emerged demonstrating the role and mechanism of ω-3 FA as specialized fat mediators, with anti-inflammatory, anti-proliferative and pro-resolving properties towards resolution of cigarette smoke-induced lung inflammation in former smokers. Research has 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, research staff hypothesize that a standardized formulation of CUR + ω-3 FA will target molecular pathways that are critical for lung cancers development, leading to a reduction in the overall size and density of nodules, in former smokers. The hypothesis is this will be mediated by reducing inflammation and through pro-resolving effects in the nodules. Their research staff will test this hypothesis by using an experimental design and rigorously evaluating the safety, efficacy and validate the potential mechanism of a combination of ω-3 FA + CUR or placebo administered for six months in former smokers, age ≥55 years, with lung nodules detected during LDCT screening program. Results of the proposed trial may have immediate and significant benefit to former smokers and other high-risk populations towards lung cancer prevention. The goal is to obtain the safety and effectiveness of the combination of ω-3 FA + CUR or placebo in 100 men and women who are diagnosed with the lung nodules. As proposed in the study timeline, the initial three months of the study have been to complete most of the clinical trial start up activities. The staff has received approval to start the trial from the Moffitt Cancer Center Scientific review committee. Approval to use the combination of ω-3 FA + CUR or placebo as an investigational drug in this patient population with lung nodules was received from the United States Food and Drug Administration (US FDA). The full trial has been submitted to the institutional review board (IRB) for full approval. Staff have been hired and trained and will be working with the investigators in this trial. All the Quit lines in the State of Florida have been contacted to promote the clinical trial, once it is approved. The research staff have scheduled a meeting with all the investigators and smoking cessation program staff to make sure that everything is ready to implement the full trial as soon as approval (IRB) is received.

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 J. Kaye, MD

   Organization: University of Florida

   Abstract of Proposed Research: Small cell lung cancer (SCLC) is an aggressive subtype of lung cancer that rapidly metastasizes to distant organs for which there have been no improvements in standard treatments for past 35 years. Therefore, there is a great need for new strategies. Dr. Kaye and associates previously hypothesized that oncolytic virotherapy using myxomavirus (MYXV) could serve as an innovative treatment for tumor cells resistant to standard chemotherapy. They also hypothesized that oncolytic MYXV could complement and synergize with new systemic immunotherapy strategies that activate host immune cell killing. The researchers have now generated strong preclinical data demonstrating efficient MYXV infection, late viral replication, and marked cytotoxicity in human and mouse SCLC tumor cells in vitro and in both immunocompetent genetically engineered SCLC mouse models and in patient derived xenografts from biopsies of newly diagnosed SCLC patients. In contrast, there was no toxicity to human and mouse normal cells in vitro or in vivo. These observations have generated support from DNAtrix that has licensed MYXV for human clinical studies. They now propose an ambitious clinical phase 1 trial testing the safety and efficacy of intratumoral delivery of MYXV by

navigational ultrasound-guided bronchoscopy. Research Aim 1 outlines the tasks and goals to generate 'good-manufacturing-practice' (GMP) clinical grade MYXV. Research Aim 2 outlines a phase 1 clinical trial with objectives of i) patient safety during MYXV dose escalation via intratumoral delivery; ii) assessment of objective tumor response within target nodal/tumor mass that received intratumoral MYXV and iii) exploratory objectives to test susceptibility of fresh SCLC samples collected at the initial biopsy to MYXV cytotoxicity in vitro and compare this data with outcome of matched patients receiving intratumoral MYXV delivery in vivo. They will also track MYXV DNA in blood and sputum pre- and post-treatment and will perform studies for biomarkers on tumor necrosis and immune activation. This is a newly awarded grant executed in 2018 and over the past months the researchers have submitted formal collaborative agreements between the University of Florida and DNAtrix, Inc as well as formal agreements between University of Florida and Arizona State University and Oncomyx (outlined in this quarterly progress report). They have also begun process developmental runs for harvesting MYXV to define all materials and standard process steps for the final production batch record for GMP manufacturing (outlined in this quarterly progress report).

Follow On Funding: None at the time of reporting.

Collaborations: The University of Florida Division of Sponsored Programs (DSP) is collaborating with DNAtrix, Inc.; Dr. Grant McFadden, Arizona State University; Oncomyx, Inc.; Dr. Brian Cleaver, Associate Director of the Human Applications Laboratory, Powell Gene Therapy Center; and Nissin Moussatch and Gary Brown.

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

   Abstract of Proposed Research: Several cancer types including prostate, breast, prostate, brain and lung cancers highly enriched with hyaluronan (HA). HA-mediated signaling accomplished by its interaction with cognate cell surface receptor CD44 (a non-kinase transmembrane glycoprotein). In this project, Dr. Kusmartsev and associates propose to investigate the roles of HA-mediated CD44 signaling in mechanisms of tumor-associated immune suppression. They recently reported that co-culture of bone marrow-derived myeloid cells with tumor cells promotes differentiation of myeloid precursors toward highly immunosuppressive programmed death-ligand 1(PD-L1)-expressing macrophages. The researchers have found that tumor-induced formation of PD-L1+ macrophages mediated by tumor-produced HA. Inhibition of HA synthesis in tumor cells with pharmacologic inhibitor or blockade of CD44 signaling in myeloid cells with antagonistic anti-CD44 antibody prevented formation of PD-L1+ macrophages. They also found that tumor-derived HA stimulated production of immunosuppressive and inflammatory factors IL-6, IL-10, TNF-alpha, IL-1beta and PGE2 by myeloid cells in CD44-dependent manner. These results strongly suggest an important role for tumor derived HA and HA-mediate CD44 signaling in tumor-induced immunosuppression. Their observations led them 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 (MDSCs), stimulating production of immunosuppressive factors and promoting

development of the PD-L1+ macrophages. To test the hypothesis, researchers developed aims. Aim1: Determine key molecular components involved in accumulation of immunosuppressive PD-L1-expressing myeloid cells in bladder cancer. Aim 2: Investigate whether targeting HA-CD44 link in bladder cancer could reduce tumor-associated immune suppression and improve anti-tumor immune response in tumor bearing mice and in cancer patients. Successfully accomplishing this project will define role of hyaluronic acid in immune evasion in cancer. Intervening HA-mediated CD44 signaling could offer an exciting new therapeutic approach to overcome cancer-associated immune tolerance and improve efficacy of existing cancer therapy and immunotherapy.

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

   Abstract of Proposed Research: Smoking during pregnancy is a major risk factor for spontaneous abortion, prematurity, and low birth weight. Additionally, offspring of smokers have an increased incidence of chronic behavioral problems, obesity, and nicotine addiction. Although many of the problems associated with prenatal nicotine exposure have been documented, the mechanism(s) underlying these changes remain elusive. Emerging evidence now suggests that a common factor underlying many diseases 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 dysregulation of the gut-microbiome-brain axis. At present, little is known about how smoking influences the gut-microbiome-brain axis during pregnancy and nothing is known about how prenatal exposure to nicotine modifies the gut-microbiome-brain axis in adult offspring and whether sustained gut dysbiosis contributes to a life-long predisposition for obesity, cardiovascular disease, heightened anxiety, and/or nicotine addiction in the offspring. Their preliminary analysis of the fecal samples from 21 day old rats with prenatal exposure to nicotine demonstrates there are sustained changes in the gut-microbiome. This is paralleled by alterations in the expression of genes linked to obesity and cardiovascular disease in the hypothalamus, a region of the brain associated with physiological homeostasis or balance. The primary goal of this research proposal is to evaluate for the first time the impact of prenatal nicotine exposure on the gut-microbiome during two different time points: during pregnancy and later during adulthood in the offspring. Dr. Hayward and associates hypothesize that prenatal nicotine exposure (PNE) induces changes in the maternal gut-microbiome and changes in the placental barrier, which exposes the fetus to elevated levels of microbial metabolites (short chain fatty acid [SCFAs]), hormones (leptin), and inflammatory cytokines. Moreover, disruption of the prenatal environment promotes epigenetic changes in gene expression in the offspring brain and a sustained change in the offspring gut-microbiome. These alterations linked to the gut-microbiome-brain axis promotes obesity and related diseases in adulthood. Using a pre-clinical rodent model, changes in gene expression in the fetus and adult offspring will be monitored and the effects of the maternal gut-microbiome alone versus nicotine on the overall adverse effects associated with prenatal nicotine exposure will be determined. To date they have begun the

analysis of the maternal gut microbiome relative to fetal tissues (Aim1). The researchers also propose to identify whether associated changes in the gut-microbiome of offspring are linked to the predisposition to develop high blood pressure when offspring are re-exposed to SCFAs or leptin. Finally, they will evaluate whether a simple fecal microbiome transfer can reverse or attenuate adverse health outcomes associated with prenatal nicotine. The focus of this newly awarded project is to provide new insights into potential novel and inexpensive therapies for individuals unwillingly exposed to nicotine in utero, as well as the 23% of Florida's population that are current tobacco smokers that also more likely to be obese, develop diabetes, and cardiovascular disease.

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

   Abstract of Proposed Research: Tobacco smoking is considered to be one of the major risk factors for pancreatic cancer, a disease with very poor survival rates. This disease is currently the third most frequent cause of cancer-related death in the United States with over 50,000 deaths in a year. The poor prognosis in this disease is attributed to the presence of a dense inflammatory supportive tissue (stroma) which creates a complex tumor microenvironment that is conducive to an aggressive disease. However, several therapies targeting just the stromal component have often resulted in increased growth and spreading of tumors. This suggests that targeting only stroma is not sufficient and that there is a need for an "ideal therapy" that will not only target the stromal cells but will also target tumor cells and actively prevent the tumor-stroma-immune interaction.

   Studies in their laboratory show that Triptolide, a diterpene triepoxide from the Chinese plant Tripterygium wilfordii, induces cell death in pancreatic cancer cells and is very effective in reducing tumor growth and loco-regional spread in several complementary models of pancreatic cancer. For ease of clinical application, they have now developed water-soluble pro-drug for this compound, Minnelide, which they have extensively tested against pancreatic and other cancers with very encouraging results. Minnelide has just completed Phase I clinical trial against advanced gastrointestinal malignancies and is currently awaiting Phase 2 trials. This study has yielded very encouraging results with significant tumor responses observed in terms of reduced tumor avidity on PET-CT and many patients with partial response or stable disease. This Phase I trial shows that that maximum tolerated dose for Minnelide is 0.67mg/m2. This roughly translates to 0.2 mg/kg in mice. At this dose, Minnelide depletes the stromal environment resulting in relieving the interstitial pressure on the blood vessels and leading to better drug delivery. However, at this dose the researchers observe minimal anti-tumor efficacy of Minnelide by itself. Thus, the hypothesis is if this anti-stromal low dose of Minnelide can be combined with cytotoxic therapy, it is likely to yield a robust tumor regression by facilitating drug delivery and by addressing tumor-stroma interaction.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Dauer P, Zhao X, Gupta VK, Sharma N, Kesh K, Gnamlin P, Dudeja V, Vickers SM, Banerjee S, Saluja A. (2018) Inactivation of Cancer-Associated-Fibroblasts disrupts oncogenic signaling in pancreatic cancer cells and promotes its regression. Cancer Res. 2018 Mar 1;78(5):1321-1333

Patents: None at the time of reporting.

8. **Grant #8JK08:** Reprogramming the Tumor Microenvironment in Pancreas Cancer to Enhance Immunotherapy

Principal Investigator: Nipun B. Merchant, MD

Organization: University of Miami

Abstract of Proposed Research: 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. They have previously identified a mechanistic rationale for targeting signal transducer and activator of transcription 3 (STAT3) signaling to overcome therapeutic resistance in PDAC. They have now investigated the molecular mechanisms underlying the heterogeneous response to STAT3 and RAS (*gene type*) pathway inhibition in PDAC. Effects of the Janus kinase/signal transducers and activators of transcription (JAK/STAT3) inhibition (STAT3i) or methyl ethyl ketone (MEK) inhibition (MEKi) were established in Ptf1acre/+;LSLKrasG12D/+;Tgfbr2flox/flox (PKT) mice and patient-derived xenografts (PDX). Amphiregulin (AREG) levels were determined in serum from human PDAC patients, LSL-KrasG12D/+;Trp53R172H/+;Pdx1Cre/+ (KPC) *(types of mice models),* and PKT mice. MEKi/STAT3i-treated tumors were analyzed for integrity of the pancreas and the presence of cancer stem cells (CSC). It was observed that an inverse correlation between extracellular-signal-regulated kinase (ERK) and STAT3 phosphorylation. MEKi resulted in immediate activation of STAT3, while STAT3i resulted in transarterial chemoembolization (TACE)-induced, AREG-dependent activation of epidermal growth factor receptors (EGFR) 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 amphiregulin (AREG) levels. Furthermore, MEKi/STAT3i altered the PDAC tumor microenvironment by depleting tumor fibrosis, maintaining pancreatic integrity, and downregulating CD44+ and CD133+ CSC *(phenotypes).* 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.

The research team has 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 (Tregs) 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, they 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. The results further show that combined MEKi and STAT3i with PD-1 inhibition can harness the effects of immune checkpoint

inhibitors for an enhanced anti-tumor response. Based on this data, they 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. (2018, in press) Inverse correlation of STAT3 and MEK signaling mediates resistance to RAS pathway inhibition in pancreatic cancer. Cancer Research.

Patents: None at the time of reporting.

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

Principal Investigator: Tomar Ghansah, PhD

Organization: University of South Florida

Abstract of Proposed Research: There is a relationship between tobacco smoking and pancreatic cancer (PC). The risk of getting pancreatic cancer is about twice as high among smokers compared to those who have never smoked. This study will investigate new molecular target(s) to develop effective therapeutic strategies to improve PC patient's quality of life and survival. Currently, the study is underway with hiring additional research personnel at the University of South Florida. The principle investigator and technician have surgically generated Orthotopic Pancreatic Cancer Models to evaluate anti-tumor immune responses, measure SH-2 containing inositol 5'-polyphosphatase 1 (SHIP-1) expression in immunosuppressive and effector immune cells along with performing toxicity experiments using Intraperitoneal (IP) vs. Oral Gavage (OG) injections regarding the administration of 25mg/kg of the bioflavonoid Apigenin (API). To date they have garnered some preliminary data which shows that API reduces tumor burden with no apparent toxicity in both treatments OG vs. IP. In addition, the preliminary flow cytometry data shows that API increases the mobilization of cytotoxic (CD8+) T cell to tumor microenvironment (TME) in both O.G. vs I.P. However, API-IP treated mice showed a significant difference with the mobilization of CD8+ T cells in tumor microenvironment TME compared to APIOG mice. Due to current results, the research team is planning to perform these experiments again using more mice per group (n= 7 vs. n=3) to generate statistical data from each study. They will also validate if these CD8+ T cells that mobilized to the tumor are active and are able to kill tumor targets by performing in vitro killing assays. The researchers have collected and processed other lymphoid tissues (peripheral blood-serum, bone marrow, spleens) from these pancreatic cancer animal studies and will analyze for Myeloid Derived Suppressor Cells (MDSC) and CD8+ T cell percentages, production of inflammatory cytokines along with the changes in the intracellular expression of SHIP-1 (i.e. MDSC) later this year. It is anticipated that after completion of the data analysis, the results generated from this study will be used for further research and publication studies.

Follow On Funding: None at the time of reporting.

Collaborations: There are three USF students receiving training under this project: Krystai Villalobos-Ayala (part-time technician and master's student), Ciara Alvarez (master student in the Aging and Neuroscience program) and Bradley Miller (volunteer and undergraduate student in the Chemical Engineering program).

Journals: None at the time of reporting.

Patents: Bio-Active Flavonoid Apigenin Improves Anti-PD-L1 Immunotherapy Responses in Pancreatic Cancer. Serial Number 62/609,412 Submitted 12/22/17.

APPENDIX H

FISCAL YEAR 2017-2018 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	$ 544,561.00	$ 709,192.00	3/09/2017	2/29/2020	No	No	No
7JK02	H. Lee Moffitt Cancer Center	Chung, Christine	$ 1,896,200	$ 492,762.50	$ 1,403,437.50	3/16/2017	2/28/2022	No	No	No
7JK03	University of Miami	Dietrich, W. Dalton	$ 941,589	$ 392,328.75	$ 549,260.25	3/08/2017	2/29/2020	No	Yes	No
7JK04	H. Lee Moffitt Cancer Center	Gray, Jhanelle	$ 1,895,355	$ 473,838.75	$ 1,421,516.25	3/25/2017	2/28/2022	No	No	No
7JK05	University of Florida	Jiang, Zhihua	$ 1,422,150	$ 592,562.50	$ 829,587.50	3/07/2017	2/29/2020	No	No	No
7JK07	University of Florida	Fan, Z. Hugh	$ 125,000	$ 93,750.00	$ 31,250.00	6/15/2017	12/31/2018	No	Yes	Yes

ACTIVE GRANTS FISCAL YEAR 2017-2018
(Funding Year 2016-2017)

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

   Principal Investigator: Helen Bramlett, PhD

   Organization: University of Miami

   Progress Report: Aim 1 is determining the effect of whole body vibration (WBV) on the neurobehavior of nicotine-exposed rats after stroke. In this aim, the research team will monitor the Frailty Index (FI), prior to and 1-3 months after transient middle cerebral artery occlusion (tMCAO) alone or in combination with WBV in nicotine exposed rats. The hypothesis is that WBV will reduce frailty and improve cognition in nicotine exposed rats. To test the proposed hypothesis, female rats were randomly assigned to nicotine (4.5 mg/kg/day) or saline treatment (via osmotic pump) for 16 days. On the last day of nicotine/saline exposure, rats were exposed to transient middle cerebral artery occlusion (tMCAO) for 90 min and one day after tMCAO were randomly assigned to either the WBV intervention or control group. Appropriate Sham tMCAO groups were also produced. Animals randomized to the WBV group underwent 30 days of treatment performed twice daily for 15 min each session for 5 days each week. The vibration device was programmed in order to achieve a frequency of vibration within a range of about 40 Hertz (0.3 g). Rats exposed to nicotine, tMCAO and WBV treatment were monitored for memory and spatial learning using the Morris Water Maze. At the end of WBV control exposures animals were sacrificed and brains were collected for histology. The research staff is analyzing the data for Aim 1 and are within the proposed timeframe. The results from this study have the potential to provide a translatable treatment for those individuals who smoke and have a stroke.

   Follow On Funding: None at the time of reporting.

   Collaborations: None at the time of reporting.

   Journals: None at the time of reporting.

   Patents: None at the time of reporting.

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

   Progress Report: Research Aim 1 is to determine the Mesenchymal subtype signature and T-cell receptor clonality as predictive biomarkers of Programmed Death-1 (PD-1) inhibitors and determine the tumor immune microenvironment (TIME) in a context of tobacco use in head and neck squamous cell carcinoma (HNSCC). For Aim 1a, the research team has procured detailed tobacco use history and identified formalin fixed paraffin embedded (FFPE) tumor blocks from 31 of 50 proposed HNSCC to date under the Institutional Review Board (IRB) approved protocol (MCC 18754). However, the researchers have found that the rest of the 19 tumor blocks are located at other hospitals because the biopsies or surgeries were done there and then the patients came to Moffitt for the immunotherapy treatments. The research team is in the process of

amending the IRB protocol MCC 18754 to get permission to contact the other hospitals and obtain the tumor blocks or unstained slides for analyses. Aim 1b, will analyze tissue samples collected from a phase I/II clinical trial of concurrent cetuximab and nivolumab. The clinical trial itself is being funded through Lilly Oncology while the analyses of the samples once collected are funded by this James and Esther King Biomedical Research grant. The phase I portion of the trial has been completed and currently the researchers are enrolling for the phase II portion of the trial. Sixteen patients have already been screened with 13 of the 16 enrolled. Tissue samples have been collected from all 13 patients. Aim 2 is to determine tobacco-specific genoproteomic changes that create immunosuppressive tumor immune microenvironment in current smokers reflected by a lower immunoscore compared to the never/former smokers. For Aim 2a, the researchers have completed development of the multiplex immunofluorescent staining for the immunoscore determination using the Perkin Elmer Vectra system and analysis pipeline using InForm and R package. The FFPE tumors were analyzed from 146 patients with clinical data. Immunoscore was determined by the number of CD3+ and CD8+ tumor-infiltrating immune cells in the tumor core and/or in the invasive margin of tumor. The distribution of the immunoscore across 146 samples was: Immunoscore 0, n=30; Immunoscore 1, n=25; Immunoscore 2, n=38; Immunoscore 3, n=29; Immunoscore 4, n=24. The correlations between the smoking history and the immunoscore as well as treatment response and survival are currently being evaluated.

For Aim 2b, frozen tumors were analyzed from 145 patients with clinical data. The whole exome sequencing from the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) sequencing by next generation sequencing are completed. The research team has started to determine the genomic abnormalities and correlating them with the immunoscores. Aim 3 is to develop smartphone-based assessment of patient-reported outcomes related to immunotherapy and smoking in HNSCC patients. The research team has selected study measures and formatted them for electronic administration. The study database for participant tracking has been created. The study protocol has been completed and approved by the IRB. Eighty-eight patients were screened and enrolled 37 of 100 total accrual goal 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-Squibbs as sub-contracted sites under Moffitt Cancer Center. M2GEN (Erin Siegel, PhD) is a collaborator which will increase the number of samples for immunogenomic analyses in Aim 2.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

   Principal Investigator: W. Dalton Dietrich, PhD

   Organization: University of Miami

   Progress Report: Analysis has been completed of effects of acute P7C3-A20 (a neuroprotective compound) treatment (immediately after transient middle cerebral artery occlusion (tMCAO)) in male animals on various outcomes after tMCAO. P7C3-A20-treated rats performed significantly better than vehicle-treated controls (in which animals receive treatment with the vehicle in which the experimental substance is dissolved or suspended) in sensorimotor cylinder and grid-walk

tasks, and in a chronic test of spatial learning and memory. These behavioral improvements with P7C3-A20 treatment were correlated with significantly decreased cortical and hippocampal atrophy, and associated with increased neurogenesis in the subventricular zone and hippocampal dentate gyrus subgranularzone. Furthermore, cerebral ischemia significantly reduced nicotinamide adenine dinucleotide (NAD) in the cortex but P7C3-A20 treatment restored NAD to sham (control) levels. Thus, P7C3-A20 treatment mitigates neurodegeneration and augments repair in the brain after focal ischemia, which translates into chronic behavioral improvement. This suggests a new therapeutic approach of using P7C3 compounds to safely augment NAD and thereby promote two independent processes critical to protecting the brain from ischemic stroke: mature neuron survival and postnatal neurogenesis throughout the post-ischemic brain. The researchers completed the analysis of delayed effects of P7C3-A20 treatment after tMCAO. The drug was administered immediately after reperfusion (the restoration of blood flow to an organ or tissue after having been blocked) or beginning 6hrs after reperfusion. The animals were assessed as before for behavioral improvements as well as histopathological protection. Delayed P7C3-A20 treatment significantly improved stroke-induced sensorimotor deficits in motor coordination and symmetry, as well as cognitive deficits in hippocampal-dependent spatial learning, memory retention, and working memory. In the cerebral cortex, delayed P7C3-A20 treatment significantly increased tissue sparing 7 weeks after stroke and reduced hemispheric infarct volumes 48 hours after reperfusion. Despite no reduction in striatal infarct volumes acutely, there was a significant increase in spared tissue volume chronically. In the hippocampus, only immediately treated P7C3-A20 animals had a significant increase in tissue sparing compared to vehicle-treated stroke animals. This structural protection translated into minimal hippocampal-dependent behavioral improvements with delayed P7C3-A20 treatment. However, all rats treated with delayed P7C3-A20 demonstrated a significant improvement in both sensorimotor tasks compared to vehicle controls, suggesting a somatosensory driven recovery. These results demonstrate that P7C3-A20 improves chronic functional and histopathological outcomes after ischemic stroke with an extended therapeutic window. The research team is continuing assessment of acute histopathological comparisons between males and females after tMCAO after previously establishing beneficial effects in male animals. The results from the acute and chronic treatment of P7C3-A20 are promising for being translated to the clinic. However, further preclinical testing will need to be performed.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Loris, Z.B., Pieper, A. A., and Dietrich, W. D. (2017). The neuroprotective compound P7C3-A20 promotes neurogenesis and improves cognitive function after ischemic stroke. Experimental Neurology. 290: 63 doi: 10.1016/j.expneurol.2017.01.006.

Loris, Z. B., Hynton, J. R., Pieper, A. A., and Dietrich, W. D. Beneficial effects of delayed P7C3-A20 Treatment after transient MCAO in rats. Translational Stroke Research, 1-11. doi:10.1007/s12975-017-0565-z.

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 Center

Progress Report: The purpose of this clinical trial is to build upon the existing literature and further the understanding of combination immunotherapy in Non-small cell lung cancer (NSCLC). To date, single agent immunotherapy is approved for use in second-line NSCLC treatment. In addition, the combination of immunotherapy plus chemotherapy is approved for use in newly diagnosed metastatic NSCLC. Here the research team aims to challenge this convention by removing the chemotherapy and moving immunotherapy combinations to the front-line setting. Further this trial will also investigate the use of combination immunotherapy with nivolumab, ipilimumab plus nintedanib in those patients who have progressed on prior immunotherapy. By doing a multi-compartmental approach to target the cancer cells, lymph nodes (which harbor T-cells) and the tumor microenvironment, the aim is to improve outcomes for patients with NSCLC.

The clinical protocol has been written and is now approved by Bristol Myers Squibb, Boehringer Ingelheim, the Moffitt Lung Cancer research team, Scientific review committee, Institutional Review Board as well as the Food and Drug Administration. All contracts have been executed, all orders are written and the trial has been activated since January 2018. To date, there have been 7 patients screened, treated 4, with 1 patient to start on treatment shortly. It is exciting to note that in 1 patient, significant reduction in their tumor burden was noticed. This is of particular interest as this patient had previously had tumor progression on single agent immunotherapy. The research team is following patient safety closely. Due to observed toxicity, it was felt by the Principal Investigator and research team that it was advisable to explore lower dose levels of the drug. The clinical protocol has been amended and is now submitted for review to the regulatory agencies for approval. The patients will continue to be followed closely on this trial. With the current design and findings to date, there is a high potential for impact to Floridians. In particular, the response seen in the patient with NSCLC who had previously progressed on immunotherapy is noteworthy and encouraging. The research team has a promising therapeutic approach to battle this life-threatening disease.

Follow On Funding: None at the time of reporting.

Collaborations: Dr. Sonam Puri is a hematology/oncology fellow at University of South Florida/Moffitt Cancer Center in Tampa, Florida. She is being trained in clinical trial design and execution. Her aim is the work for an academic institution as a clinical trialist.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

   Principal Investigator: Zhihua Jiang, PhD

   Organization: University of Florida

   Progress Report: This project seeks to understand the mechanisms that are responsible for exacerbation of aortic aneurysms by tobacco smoke. Aortic aneurysms are silent killers that attack tobacco users at chance six times greater than that among non-tobacco users. In 2016, the Truth Initiative released statistic data showing that 15.5% adult Floridians were or are active smokers. Therapies capable of eliminating the deleterious effects of cigarette smoke on the aortic wall will save the lives of these Floridians. Unfortunately, there is no effective medical treatments available to break the link. One of the major challenges in developing such treatments is the lack of adequate animal models that can sufficiently recapitulate the aortic phenotype of tobacco smoke. In the past year, research staff of this project have attempted to create mouse models of

aortic aneurysms using approaches combining genetic and chemical interventions. The results showed that transforming growth factor β (TGFβ) plays a critical role in maintaining structural homeostasis of the aortic wall. In mice at an adult age, selective deletion of TGFβ type II receptors (Tgfbr2iko) in smooth muscle cells leads to formation of thoracic aortic aneurysms and dissections (TAADs). This model is capable of recapitulating typical pathologies that are frequently observed in human TAADs. To determine the responsiveness of this model to cigarette smoke, the research project staff explored several ways of delivering cigarette smoke or its component to the animals. These include chronic infusion of nicotine salt or nicotine free base via an osmotic minipump implanted in the subcutaneous space, pellet-based releasing of nicotine free base, and nose cone inhalation of cigarette smoke. The dosage of nicotine and the schedule of smoking were similar to those frequently applied by the studies for pulmonary diseases. However, none of these interventions altered the aortic phenotype of Tgfbr2iko animals. Pellet–based nicotine administration significantly exacerbated the TAADs induced by Tgfbr2iko, but this approach caused acute death (in a few hours) of animals at a rate greater than 70%. These results had led the research team to hypothesize that a second hit or an extremely high pulse dosing of nicotine is required for tobacco smoke to aggravate the TAAD formation. To test the hypothesis, β aminopropionitrile (BAPN) was administered to animals as the “second hit.” The results showed that in the presence of BAPN, infusion of nicotine freebase nearly doubled the rate of aortic dilation compared to the placebo controls in which nicotine was replaced with saline. Full histologic characterization of this model is currently ongoing. With the powerful model, the research group has initiated mechanistic studies to understand how tobacco smoke impairs the immune homeostasis and to explore whether strategies to restore the immune balance can prevent the exacerbation of TAAD formation by tobacco smoke. Results to be obtained from these studies will lay a solid foundation for future projects to develop therapeutics to treat patients suffering from aortic aneurysms and reduce the risk of aneurysm formation in tobacco users.

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 #7JK07:** Laminated Paper-Based Analytical Devices for Detecting Exposure to Secondhand Smoke

   Principal Investigator: Z. Hugh Fan, PhD

   Organization: University of Florida

   Progress Report: Cigarette smoking is the leading cause of chronic obstructive pulmonary disease (COPD). Exposure of secondhand smoke (SHS) to non-smokers also induces COPD. According to the US Surgeon General Report, child exposure to SHS is associated with acute respiratory infections and childhood diseases. The American Academy of Pediatrics would like pediatricians, family doctors, and emergency room physicians to be able to tell the parents of a child with an asthma attack or respiratory infection whether SHS is causing the problem. It would be powerful for the doctors to have evidence that exposure of the child to SHS has actually occurred and to tell the parents that their smoking has had detrimental effects on their children’s health.

   The current analytical methods used for assessing exposure to SHS are based on specific

biomarkers of tobacco smoke. The biomarkers can be measured by approaches including immunoassays, high-performance liquid chromatography (HPLC), and mass spectrometry. However, these methods require specialized instruments that are too bulky to be placed at the point of care. The experimental procedures are often labor-intensive and time-consuming, leading to lengthy and costly analysis.

To address the challenge, research staff have been developing laminated paper-based analytical devices (LPAD) for detecting SHS exposure. LPAD are developed by borrowing the concept from pH papers and pregnancy test strips. They are of low-cost, easy to operate by nontechnical personnel, and manufacturable.

The SHS detection method of LPAD is based on the presence of cotinine, the primary metabolite of nicotine in bodily fluids (e.g., blood, urine, and saliva). Using the format of competitive immunoassay, unknown amount of cotinine in a sample will compete with known amount of cotinine-HRP (horseradish peroxidase) for anti-cotinine (i.e., cotinine antibody) immobilized on the surface of LPAD. The amount of HRP detected afterwards can be correlated to the level of cotinine in the sample.

Research staff have also studied a couple of enrichment methods, which are required for concentrating biomarkers in samples so that the assay is sensitive enough to detect SHS exposure. One enrichment method is to take advantage of evaporation in a field environment or in a lab. Research staff demonstrated the concentration effects of this method in a paper strip. In addition, they have explored in using sessile droplets for sample enrichment. Since a droplet on a post is in 3 dimensions (3D), it has much higher surface area than a flat surface in 2D. As a result, a droplet has much higher evaporation rate. Research staff have designed various devices based on this concept and studied their enrichment factors.

After the validation of such a device in the future, the devices will be available for studying the impacts of SHS exposure in a variety of environments, among a range of population, as well as for longitudinal investigation. Those who will benefit from the research include children as well as the general population as discussed above.

Follow On Funding: National Science Foundation - $50,000

Collaborations: None at the time of reporting.

Journals: Cassano, C. L., Georgiev, T., Fan, Z. H, “Using Airbrushes to Pattern Reagents for Microarrays and Paper-fluidic Devices," Microsystems & Nanoengineering, 2017, 3: 17055; doi:10.1038/micronano.2017.55.

Jiang, X; Pan, M; Loeb, J.; Lednicky, J.; Wu, C.Y.; Fan, Z.H. “Flu Virus Aerosol Collection and Paper-based Viral RNA Detection”, in Proceedings of the 21st International Conference on Miniaturized Systems for Chemistry and Life Sciences (μTAS’2017), 2017: 583-584.

Patents: None at the time of reporting.

APPENDIX I

FISCAL YEAR 2017-2018 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	Wei, Sheng	$ 1,231,336	$ 957,705.74	$ 273,630.26	3/19/2016	2/28/2019	Yes	No	No
6JK02	H. Lee Moffitt Cancer Center	Drobes, David J.	$ 1,186,164	$ 553,543.20	$ 632,620.80	3/19/2016	2/28/2021	No	No	No
6JK03	University of Florida	Liao, Daiqing	$ 795,236	$ 614,854.72	$ 180,381.28	3/09/2016	2/28/2019	Yes	Yes	No
6JK04	Florida International University	Miguez, Maria Jose	$ 1,628,449	$ 773,337.87	$ 855,111.13	3/19/2016	2/28/2021	No	Yes	No
6JK06	H. Lee Moffitt Cancer Center	Park, Jong Y.	$ 1,231,336	$ 957,705.74	$ 273,630.26	3/21/2016	2/28/2019	No	No	No
6JK08	Florida Atlantic University	Wu, Jang-Yen	$ 1,231,336	$ 957,705.74	$ 273,630.26	3/31/2016	2/28/2019	Yes	Yes	No

ACTIVE GRANTS FISCAL YEAR 2017-2018
(Funding Year 2015-2016)

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

   Principal Investigator: Sheng Wei, MD

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: It is well established that natural killer (NK)-mediated anti-tumor responses should be a key component of future immunotherapeutic applications. However, study of NK cells in murine models have been hindered by the ability of NK cells to reach its intended target. In particular, this 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 (TGFβ) secretion in H1299) of the cancer cells. In vitro, the researchers have developed a 3D hydrogel model that aids in the analysis of cytokine production kinetics from tumor cells and their effect on trafficking NK cells which serve 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 NSG (NOD/SCID/ c c –/–) tumor-bearing mice but to traffic into the tumor, once established, allowing tumor growth. The most recent work demonstrates that enhancement of NK cell in-tumor trafficking is mediated by the chemokine, CX3CL1. In this regard, this work contributes to the future study of NK cells in tumor by overcoming NK cell survivability and in-tumor restriction in vivo through supplementation of NK cells with the cytokine IL15 and the increase in CX3CL1 ligand overexpression in the murine tumor model.

   The most recent data demonstrate the combined injection of IL-15, which aids in increasing the survival of NK cells, and overexpression of CX3CL1 in tumor cells, which allows NK trafficking into the tumor, gives a comprehensive model to study intra-tumoral NK cells in preclinical applications including planned studies with the research team's preloaded nanoparticles. Moreover, thus far the study has provided a clear rationale as to why NK cells may be excluded in vivo from the tumor site which will be further explored as the research moves forward in testing the anti-miR183 or TGFb anti-sense MnO_2 nanoparticles in the last part of the study. The research team believes that these studies thus far are impactful to the community by: providing more information as to the obstacles posed to NK cells by the tumor, establishing an in vivo model to study future therapies aimed at improving NK cell function for therapeutic use, and allow the testing of the team's novel therapeutic delivery of payloads in nanoparticles to NK cells for enhancement of their function and overcoming tumor-mediated immunosuppression. It is believed that the funding for this study provided a clear basis for this development and will be the basis for several published reports and follow on grant funding as demonstrated by the close score in a NIH grant by one of the investigators of this research. It is expected that the investment of Floridians on this work will not only benefit cancer patients in the state but will be the foundation for future work on NK cells that can lay the foundation for future therapeutic development.

   Follow On Funding: None at the time of reporting.

   Collaborations: None at the time of reporting.

   Journals: None at the time of reporting.

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

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

   Principal Investigator: David J. Drobes, PhD

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: This project is developing and testing a novel smoking cessation intervention that utilizes reduced nicotine content cigarettes prior to quitting, along with targeted self-help treatment materials and individual counseling. The overall purpose is to determine if this smoking cessation strategy is efficacious, and whether an immediate or gradual transition to very low nicotine content cigarettes during the pre-quit period is optimal.

   During the current reporting period, the research project staff finalized all study materials and obtained approval from the University of South Florida (USF) Institutional Review Board to conduct the pilot study (Study 1). Subsequently, participant recruitment and data collection for the pilot study was initiated and completed. Pilot data were reviewed on an ongoing basis, in order to enhance study materials and procedures for a subsequent randomized controlled trial (RCT; Study 2; n=208). More specifically, feasibility was measured by collecting feedback from study participants and project staff regarding the study intervention workbook and other aspects of the treatment. All feedback was reviewed by the research team, including the PI, Co-Investigators, study consultants, and Research Coordinators. Based on the pilot study feedback and review process, the following enhancements and adjustments are planned for the RCT (Study 2):

   The workbook utilized in the pilot study has been converted to a series of weekly booklets. This will serve as an additional incentive for research participants to attend weekly appointments (i.e., in order to receive new treatment materials), and to avoid participants from working ahead in the single (larger) booklet. In addition, the study team has contracted with a graphic design company to make the treatment booklets more visually appealing and user-friendly.

   Cognitive-behavioral smoking cessation assistance will be provided throughout the pre-quit treatment period, rather than strictly during the final week. An additional visit will be added on the participants' Target Quit Day (TQD), to facilitate the transition from smoking low-nicotine cigarettes to complete abstinence.

   In addition to the refinement of the targeted intervention, the research project team observed that a larger than expected proportion of individuals screened for the pilot study were deemed ineligible; accordingly, several eligibility criteria were adjusted for the upcoming randomized controll trial (RCT), in order to expedite recruitment and allow for a more generalizable sample. Specifically, the study team loosened some of the medical exclusion criteria, allowed for greater use of other tobacco products, and permitted more recent nicotine replacement therapy use prior to study enrollment. The aforementioned modifications were reviewed and approved by the study team licensed medical professionals.

   The project is currently transitioning from the pilot phase to the larger RCT. Accordingly, data are not yet available to determine the actual impact to Floridians. However, 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: The University of South Florida has two undergraduate students (Briana Merkher and Brooke Sprague) receiving training.

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

   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 2018, about 266,120 new cases of breast cancer are expected to be diagnosed in women in the U.S. 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 is still poor. Therefore, development of new therapy for advanced breast cancer is urgently needed to improve treatment outcomes for patients with advanced breast cancer. CREB (cyclic amp-response element binding protein) binding protein (CBP) and its homologue p300 are increasingly recognized as therapeutic targets for breast cancer. The goal of this grant is to understand the roles 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. 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: Currently, four Ph.D. students and nine undergraduate students at University of Florida are performing research in the Principle Investigator's laboratory.

   Journals: Li D, Tian G, Wang J, Zhao LY, Co O, Underill ZC, Mymryk JS, Claessens F, Dehm SM, Daaka Y, Liao D. (2018). Inhibition of androgen receptor transactivation function by adenovirus type 12 E1A undermines prostate cancer cell survival. The Prostate. 1–17. doi:10.1002/pros.23689

   Patents: U.S. Provisional Patent Application entitled Polypeptide Inhibitor of De Novo Lipogenesis in Cancer Cells. Serial No.: 62/653,183. Filing Date: 04/05/2018. Inventors: Daiqing Liao, Iqbal Mahmud, Guimei Tian UF#-17034 (222110-8200)

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

   Principal Investigator: Maria Jose Miguez, MD, PhD

   Organization: Florida International University

   Progress Report: Though Florida lags behind in reducing the rates of tobacco use, it will save approximately $8.2 billion in smoking-related health care costs associated with smoking cessation

programs by 2020. Targeting tobacco cessation programs to groups with higher smoking rates will be highly beneficial. On average, 15% of the adults in Florida smoke, for people living with human immunodeficiency virus (HIV), the situation is even worse as their smoking rates are 40-80% and they are more likely to develop serious tobacco conditions (i.e. cancer, heart and lung disease) than those without HIV. Indeed, nowadays HIV positive individuals are more likely to die from tobacco-related disease than from HIV. Of concern, there is a paucity of controlled trials targeting people living with HIV, and in the existing research, cessation rates are sub-optimal. Ten prior randomized trials conducted with people living with HIV yielded quit rates ranging from 4% to 14%. Those rates were obtained despite using different intervention approaches, such as cell-phone counseling, motivational interviewing, alone or in combination with pharmacotherapy.

In this ongoing clinical trial, smoking status was assessed both as seven-day point prevalence abstinence ("Have you smoked at all, even a puff, in the last seven days?") and continuous abstinence (smoking at all since the target quit day).

Quitting rates reached 16% for the standard arm versus 18% in the tailored arm at 12 months. These rates are extraordinary when compared to the limited success exhibited by prior studies. Therefore, these interventions are not only reaching a large population in need (it will target 500 individuals), they are maximizing smoking cessation in the context of HIV. Finally, those that have not been able to quit have significantly reduced their smoking rates.

Follow On Funding: None at the time of reporting.

Collaborations: This project is collaborating with Dr. Castro, the University of Miami study physician. The study procedures are administered at the Clinical Research Center, which is staffed by professionals with experience in clinical research involving people living with HIV. The study is also performing laboratory testing at the University of Miami since the laboratory is taking part in the national (NIH, CDC) external quality proficiency testing programs**.**

Journals: Maria Jose M, Clery Q, Calonie G, Diego B, Castro G, Caroline P, Luis E. (2018). Gender and racial differences in enrollment and follow-up in a smoking clinical trial targeting people living with HIV: Implementation and preliminary outcomes. Int J HIV AIDS Res. 1:1 (12-16).

Patents: None at the time of reporting.

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

   Principal Investigator: Jong Y. Park, PhD

   Organization: H. Lee Moffitt Cancer Center

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

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

   The proposed project will lead to the development of an extremely valuable research asset for health disparity studies for prostate cancer. This resource will contribute not only generate important scientific findings but also allow researchers to leverage additional national funding,

such as National Institutes of Health (NIH), or Department of Defense (DOD) and ultimately lead to better strategies to reduce prostate cancer incidence and mortality.

Based on responses from the patients, the second mail package containing an informed consent and a brief questionnaire was sent to 600 patients. The research team is waiting responses from 281 patients while 270 patients returned the signed informed consent forms and completed questionnaires. Once the signed informed consent is received, the third package including a saliva collection kit and a medical release form was sent to the participants with a $10 check. So far, saliva kits to 268 patients have been mailed out with 210 saliva samples received. Based upon previous experiences, it is anticipated 1,000 saliva samples will be collected.

Follow On Funding: None at the time of reporting.

Collaborations: This is a collaboration with the University of Miami/Sylvester Cancer Center and the UF Health Cancer Center in Jacksonville.

Journals: None at the time of reporting.

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

   Progress Report: This research project is to determine which Granulocyte Colony-Stimulating Factor (GCSF) gene vectors that the research team designed provides the best results in cell-based or animal- based stroke models based on the molecular and cellular biomarkers and functional tests. The molecular and cellular biomarkers used for cell-protection and cell survivals include B-cell lymphoma-2 (Bcl-2), protein kinase B (Akt), Phosphorylated protein kinase B (p-Akt) and Optic Atrophy 1 (OPA1) and those used for cell stress/injury and cell death include BCL2-associated X protein (BAX), 78-kDa glucose-regulated protein (GRP78), dynamin-related protein 1 (DRP1) and Bcl-2-homology (BH)-3 domain only protein (Beclin 1). The highlights of the progress made during this period are summarized as follows: Development of a new method for delivery of Adeno-associated virus-human granulocyte colony stimulating factor (AAV-hGCSF) gene vectors to mice via eye drop method and demonstration of the expression of hGCSF messenger ribonucleic acid (mRNA) in the brain. The presence of hGCSF mRNA in the brain after infection with Adeno-associated virus-granulocyte colony stimulating factor-cytomegalo-virus (AAV-CMV-GCSF) gene therapy was confirmed using real time Polymerase chain reaction (PCR), quantitative real-time polymerase chain reaction (qRT-PCR). In addition, the expression of human GCSF protein (hGCSF) was confirmed by immunoblotting test using specific antibodies against hGCSF. 2. Demonstration of the efficacy of the delivered GCSF gene therapy in cell-based stroke model, namely, protection of hypoxia-induced cell injury in phaeochromocytoma (PC-12) cells by AAV-CMV-GCSF, Adeno-associated virus-human synapsin I-granulocyte colony stimulating factor (AAV-SYN-GCSF), Adeno-associated virus-hypoxia response elements-human synapsin I-granulocyte colony stimulating (AAV-HRE-SYN-GCSF) gene vectors. 3. Demonstration of the efficacy of the delivered GCSF gene therapy in animal stroke model by showing a reduction of stress markers for endoplasmic reticulum (ER), e.g., GRP78, Activating transcription factor 4 ATF4, for pro-cell death marker, e.g., BAX, for autophagy marker, e.g., Beclin-1 and for mitochondrial marker, DRP1 and an increase of pro- cell survival markers e.g., Bcl-2, pAkt and enhancer for mitochondrial functions e.g., OPA1 in addition to behavioral

functional test, namely, the locomotor activity test. The locomotor activity was found to be significantly improved by GCSF gene therapies with a rank order of AAV-HRE-SYN-GCSF > AAV-SYN-GCSF > AAV-CMV-GCSF which is in general consistent with the results obtained from molecular biomarkers analysis. This supports the hypothesis that presence of the hypoxia-response promoter (i.e., HRE) is critical for a stroke gene therapy. 4. Elucidation of the mechanism of AAV-GCSF gene therapy in BCAO stroke model including both neuro-protection and neuro-genesis. The neuro-protective mechanism is supported by a reduction of cell stress markers and an increase of pro-cell survival markers after GCSF gene therapy as stated above in 3. The neurogenesis mechanism is supported by the observations that an increase of cancer antigen protein (Ki 67), a marker of cell proliferation, and Nestin, a marker for neural stem cells/ neural progenitors were obtained using immunohistochemical localization after GCSF gene therapy. Both Ki67 and Nestin positive cells increase in bilateral carotid artery occlusion (BCAO) stroke mice model after administration of GCSF gene therapy suggesting that G-CSF gene therapy further enhances the proliferation of neural stem cells in the BCAO model.

Follow On Funding: None at the time of reporting.

Collaborations: This is a collaboration with Dr. Yunging Kang, Assistant Professor, Department of Ocean and Mechanical Engineering, Florida Atlantic University, Boca Raton, FL. Doctoral student Enze Qian, Ph.D. is studying in the College of Engineering and Computer science, FAU.

Journals: Jong, C. J., Ito, T., Prentice, H., Wu, J.Y., and Schaffer, S.W. (2017). Role of mitochondria and endoplasmic reticulum in taurine-deficiency-mediated apoptosis. Nutrients 9(8): 795 doi:10.3390/nu9080795

Modi, J., Altamimi, A., Morrell, A., Chou, H., Menzie, J., Weiss, A., Marshall, M.L., Li, A., Prentice, H., and Wu, J.Y. (2017). Protective functions of AEURA in cell based model of stroke and Alzheimer disease. J. Neurosci. Neurol. Disord. 1: 016-023

Prentice, H., Pan, C., Gharibani, P.M., Ma, Z., Price, A.L., Giraldo, G.S., Retz, H.M., Gupta, A., Chen, P.C., Chiu, H., Modi, J., Menzie, J., Tao, R., Wu, J.Y. (2017). Analysis of neuroprotection by taurine and taurine combinations in primary neuronal cultures and in neuronal cell lines exposed to glutamate excitotoxicity and to hypoxia/re-oxygenation. Adv. Exp. Med. Biol. 975: 207-216 doi:10.1007/978-94-024-1079-2_18

Prentice, H., Gharibani, P.M., Ma, Z., Alexandrescu, A., Genova, R., Chen, P.C., Modi, J., Menzie, J., Pan, C., Tao, R., Wu, J.Y. Neuroprotective functions through inhibition of ER stress by taurine or taurine combination treatments in a rat stroke model. (2017). In: Lee DH., Schaffer S., Park E., Kim H. (eds.) Taurine 10. Advan. Exper. Med. Bio. 975: 193-205 doi:10.1007/978-94-024-1079-2_17

Howard P, Jang-Yen W, Andrew W, Michael LM. (2018). AEURA, a novel homeopathic agent, shows high level protection against viral infection and stress induced neuronal toxicity. J Biomed Sci Appl. 2(1:3)

Li L, Devin W.M, Desislava D., Brandon J.D., Paul R.K., John H.Z., Jiping T. (2015). G-CSF attenuates neuroinflammation and stabilizes the blood-brain barrier via the PI3K/Akt/GSK-3β signaling pathway following neonatal hypoxia-ischemia in rats. Experimental Neurology. 272. 10.1016/j.expneurol.2014.12.020

Biswal, M.R., Prentice, H.M., Smith, G.W., Zhu, P., Tong, Y., Dorey, C.K., Lewin, A.S., Blanks, J.C. (2018). Cell-specific gene therapy driven by an optimized hypoxia-regulated vector reduces choroidal neovascularization. J Mol Med. doi:10.1007/s00109-018-1683-0

Patents: The patent entitled **“**Carbamathione, S-(N,N-diethylcarbamoyl)glutathiones, as a novel agent for the treatment of stroke”; U.S. Provisional patent application filed: FAU Ref. No.: 201708 FOX Ref. No.: 6818-298-PRO (157457.03600); Date filed: 03/08/2018; Inventors: Jang-Yen Wu

APPENDIX J

FISCAL YEAR 2017-2018 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	$ 1,464,750.00	$ 488,250.00	5/25/2015	5/15/2019	No	Yes	No
5JK02	University of Miami	Campos, Michael	$ 1,951,531	$ 1,170,918.60	$ 780,612.40	5/25/2015	5/15/2020	No	Yes	Yes
5JK03	H. Lee Moffitt Cancer Center	Simmons, Vani N.	$ 1,904,351	$ 1,133,810.60	$ 770,540.40	5/25/2015	5/15/2020	No	Yes	Yes
5JK04	University of Florida	Kaye, Frederic J.	$ 1,414,858	$ 1,294,933.89	$ 119,924.11	5/25/2015	5/31/2019	No	No	Yes
5JK05	University of Florida	Ostrov, David	$ 1,464,750	$ 1,375,107.77	$ 89,642.23	5/25/2015	11/30/2018	Yes	No	Yes
5JK06	H. Lee Moffitt Cancer Center	Cress, William D.	$ 1,145,378	$ 1,049,929.83	$ 95,448.17	5/25/2015	11/30/2018	No	Yes	Yes

ACTIVE GRANTS FISCAL YEAR 2017-2018
(Funding Year 2014-2015)

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

   Principal Investigator: David J. Lee, PhD

   Organization: University of Miami

   Progress Report: Smoking tobacco is steadily related with mortality and morbidity, from health conditions such as heart disease, diabetes, respiratory illnesses, and cancer. Prevalent health disparities associated with smoking exist among socioeconomic status groups (SES) 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 exam 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. Second, this study will be the first to explore the relationship between hypothalamic pituitary adrenal axis (HPA) functioning and race/ethnicity among treatment-seeking smokers. Cortisol 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. Third, 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. This study 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.

   The research staff seeks to hire and train staff, continue, active participant recruitment, conducted smoking cessation intervention groups for both treatment conditions, completed baseline, end-of-therapy, and three, six, and 12 month assessments. The project's timeline cohort goals are surpassed and recruitment efforts are ahead of schedule. Given the design of this longitudinal RCT, the staff intends to enroll comparable proportions of the three-major racial/ethnic groups in the U.S. – Whites, African Americans, and Hispanics. Over the last year, the research team revised the recruitment strategies to include targeted social media campaigns, public transportation advertisements, and held intervention sessions in regional cancer center locations (in both South Florida and Central Florida) to increase accessibility for non-Hispanic Whites and Hispanics (attracting African American smokers at the main cancer center locations as been successful). Both the University of Miami (UM) and Moffitt Cancer Center (MCC) sites continued to focus on the issue of enrolling non-Hispanic Whites and Hispanics by implementing new recruitment tactics in order to recruit and retain approximately equal proportions across all three demographics. The study team also closely monitored each of the study milestones, data quality, and moderately aggressive retention efforts. The scientific direction of the study remained the same. The research team continues analysis of baseline data to study racial/ethnic differences in perceived discrimination and relationship to overall smoking quit attempts.

   Follow On Funding: None at the time of reporting.

   Collaborations: None at the time of reporting.

Journals: Hooper, M.W., Lee, D.J., Simmons, V.N., Brandon, K.O., Antoni, M.H., Unrod, M., Asfar, T., Correa, J.J., Koru-Sengul, T., Brandon, T.H. (2018) Reducing racial/ethnic tobacco cessation disparities via cognitive behavioral therapy: design of a dualsite randomized controlled trial. Contemporary Clinical Trials. 68, 127-132. doi:10.1016/j.cct.2018.03.017

Patents: None at the time of reporting.

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

   Principal Investigator: Michael Campos, MD

   Organization: University of Miami

   Progress Report: In healthy persons, the lungs and airways are cleared from dust, viruses and bacteria to prevent disease development. Cigarette smoke impairs these host defense systems, allowing mucus build up, revealed by cough productive of phlegm. This is associated with frequent infections and possible destruction of the lung, diseases called chronic bronchitis and Chronic Obstructive Pulmonary Disease (COPD). From a public health perspective, smoking cessation is therefore an important goal. To try to decrease nicotine craving during smoking cessation, tobacco-free nicotine delivery devices such as electronic cigarettes (ECs) are used. However, the safety of inhaled nicotine via ECs is unknown. Over the last year, the research team investigated the possible damage from EC vapor to human cells that represent the airway surface in a dish. The results show that vapor causes changes in these cells with a reduction in hydration of the surface which results in a decreased ability to clear the airways from injurious molecules, similar to cigarette smoke. In addition, vapor causes inflammatory reactions of these cells. Many of these effects are mediated by a receptor that has not previously been linked to adverse effects of vapor in airway epithelia. These cellular experiments were confirmed in human subjects who vaped: examination of their nasal secretions revealed development of inflammation upon vaping within a week. The research team also tested whether the strategy to quit smoking with ECs is safe. While the inflammation caused by smoking is reduced upon replacing tobacco cigarettes with EC vaping within 16 weeks, it does not disappear completely. In addition, the craving for nicotine is more difficult to satisfy with EC vapors since delivery of nicotine to the blood stream requires adaptions in vaping behavior, possibly exposing the airway to more vapor than previously believed with the consequence of detrimental effects on the ability to clear dust, viruses and bacteria and mucus build up. The outcomes of this project are therefore important for subjects with smoke-induced lung diseases, but also provide a decision-making basis for subjects and policy makers about how to use and regulate nicotine delivery devices such as ECs.

   Follow On Funding: Flight Attendant Medical Research Institute - $325,500; National Heart, Lung, and Blood Institute - $179,298

   Collaborations: This project is in collaboration with Juan Sabater, Mount Sinai Medical Center; Robert Foronjy and Pat Geraghty, Downstate University NY.

   Journals: Krick S., Grabner A., Baumlin N., Yanucil C., Helton S., Grosche A., Sailland J., Geraghty P., Viera1 L., Russell1 D.W., Wells J.M., Xu X., Gaggar A., Barnes J., King G.D., Campos M., Faul C., and Salathe M. (2018). Fibroblast Growth Factor 23 and Klotho Contribute to Airway Inflammation. Eur. Respir. J. 52:(1). pii: 1800236. doi: 10.1183/13993003.00236-2018.

   Guerrero-Cignarella A., Diaz L.V.L., Balestrini K., Holt G., Mirsaeidi M., Calderon-Candelario R., Whitney P., Salathe M., Campos M.A. (2018). Differences in vaping topography in relation to adherence to exclusive electronic cigarette use in veterans. PlosOne. 13(4):e0195896 doi:10.1371/journal.pone.0195896.

Patents: None at the time of 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

   Progress Report: The goal of this project 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.

   Previously, Aim 1 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 National Cancer Institute e booklet (Aim 2) is ongoing. Assessments are conducted every six months for two years.

   Currently, recruitment for the RCT has been completed, with 881 Hispanic smokers screened and 555 participants eligible, enrolled, and returned baseline assessments. Additionally, administration of the six-month follow-up assessment was also completed. Follow-up assessments for 12 and 18 months were created and administrations are ongoing. Bioverification (carbon monoxide) testing to verify abstinence has begun and is ongoing. A 24-month follow-up assessment has been created and will begin being administered in November 2018. The completed baseline and six-month follow-up data are being coded and readied for analyses.

   Follow On Funding: National Cancer Institute - $2,839,022

   Collaborations: None at the time of reporting.

   Journals: Piñeiro, B., Díaz, D.R., Monsalve, L.M, Martínez, Ú., Meade, C.D., Meltzer, L.R., Brandon, K.O., Unrod, M., Brandon, T.H., Simmons, V.N. (2018). Systematic transcreation of self-help smoking cessation materials for Hispanic/Latino smokers: Improving cultural relevance and acceptability. Journal of Health Communication, 23(4): 350-359. doi:10.1080/10810730.2018.1448487. PMID: 29533167

   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 J Kaye, MD

   Organization: University of Florida

   Progress Report: Small cell lung cancer (SCLC) is a particularly aggressive subtype of lung cancer that was recently 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. 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. This includes recent clinical trials that show only a modest tumor response for new immunotherapy regimens in this disease. Oncolytic viral therapy has been proposed as an innovative anti-cancer and immune stimulatory treatment strategy for solid tumors that are resistant to immunotherapy alone. However, there is a need to determine safety and to optimize host immune activation using preclinical immune competent animal models in previously untested common adult tumors. The research team has now studied a modified oncolytic myxoma virus (MYXV) that shows high efficiency for tumor-specific cell death in SCLC. A specialized SCLC genetically engineered mouse model has demonstrated safety of intrapulmonary delivery of MYXV with efficient tumor-specific viral replication and cytotoxicity associated with marked host immune cell infiltration that was sustained over 60 days. There was a statistically significant increase in murine SCLC survival following intrapulmonary MYXV that was enhanced by combined low-dose cisplatin chemotherapy as compared to cisplatin alone or saline control. The researchers also tested intratumoral delivery of MYXV using a different immune competent mouse tumor model and observed acute immune cell infiltration associated with SCLC tumor necrosis. Freshly collected primary human SCLC tumor cells were permissive to MYXV infection and intratumoral MYXV delivery into patient-derived SCLC xenografts which resulted in extensive tumor necrosis. These studies support MYXV as a safe and effective anti-cancer and immune enhancing therapy to improve the outcome for patients with advanced SCLC. The research team is now completing studies to determine the optimal scheduling and dosing for combining MYXV viral therapy with new immunotherapy checkpoint inhibitors in preparation for a phase 1 clinical trial in patients with advanced lung cancer.

Follow On Funding: Florida Department of Health (James and Ester King Biomedical Research Program) - $1,360,857

Collaborations: This project is collaboration between Departments of Medicine, Molecular Genetics & Microbiology, and Anatomy and Cell Biology within the University of Florida (UF)College Medicine. Daniel Shabashvilli is a postdoctoral fellow working on this project. Patrick Kellish is a UF BMS graduate student who is working on human PDX models for small cell lung cancer and also immunocompetent allograft mouse models to optimize immunotherapy activation with oncolytic virotherapy for lung cancer. University of Florida undergraduate students are working on this project under course work BMS 4905 credit. Connor Hertzell, undergraduate student received Research Scholar award from UF to work on this project. Future collaboration with DNAtrx (Houston, TX) and the UF GMP manufacturing facility is planned to generate MYXV for human phase 1 clinical trial. Also, collaboration with the Tumor Immunology Program at Moffitt Cancer Center is planned if a clinical trial with combined anti-PD1/CLA4 plus MYXV virotherapy is granted.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

5. **Grant #5JK05:** Novel Small Molecules for Alpha-1 Antitrypsin Deficiency

   Principal Investigator: David Ostrov, PhD

   Organization: University of Florida

   Progress Report: Genes encoding the protein alpha-1 antitrypsin (AAT) contribute significantly

to risk of chronic obstructive pulmonary disease, liver cirrhosis and liver cancer. In particular, a form of AAT gene termed Z-AAT promotes pathogenesis because of a mutation that destabilizes the protein, causing it to aggregate in hepatocytes and promote disease. The focus of this project is to test a central hypothesis: specific small molecules that inhibit alpha-1 antitrypsin aggregation can be identified and developed into novel therapeutic drugs to reduce risk of alpha-1 antitrypsin deficiency associated liver diseases (e.g., liver cirrhosis, liver cancer). Previously, significant progress was achieved on each research Aim.

Aim 1: To identify more effective lead compounds through an in silico molecular docking approach and functional cell culture system. The research team identified an active candidate compound by high throughput molecular docking (4','5-(Methylenedioxy)-2-Nitrocinnamic Acid Compound 1) and a cell-based functional system. The researchers then screened structure analogues and identified 5-methyl-3-[(6-nitro-2H-1,3-benzodioxol-5-yl) methylidene] oxolan-2-one (Compound 3) which was more effective in vitro (reducing intracellular ZAAT aggregation).

Aim 2: To improve the efficacy of the lead compounds on interfering with ATZ polymer formation and ATZ secretion through computer-assisted molecular optimization to generate new derivatives. The first Z-AAT crystal structure was recently solved (PDB code 5IO1), suggesting that a mutation at position 342 destabilizes the Z-AAT molecule, thereby promoting polymerization and pathogenesis. The team screened 139,735 drug-like small molecule compounds by molecular docking to identify compounds that stabilize the E342K structural site on Z-AAT. Also screened was a novel indole ring distortion library of 180 small molecules generated by Dr. Huigens (a professor in UF College of Pharmacy) by targeting at the E342K site. 5 compounds from the National Institutes of Health Developmental Therapeutics Program and 2 compounds from Dr. Huigens' collection were shown to decrease intracellular Z-AAT levels, not wild-type AAT levels, without toxic effects on cells. The albumin protein levels were not changed after the former 5 compounds treatment, demonstrating specificity for Z-AAT.

Aim 3: To test the effectiveness of the lead compounds in AAT transgenic animal model. Earlier, lead compounds (compound 1 and compound 3) were tested for effects in transgenic mice expressing human Z-AAT (referred to as PiZ mice). Reduced Z-AAT protein levels were observed in the compound 1 treated group. To researchers' knowledge, this is the first drug-like molecule shown to function in vivo to inhibit the aggregation of Z-AAT in hepatocytes. A manuscript was submitted with these data to the high impact journal Hepatology, which has encouraged submission of a revised version. The reviewers requested analysis of fibrosis in treated animals. Since fibrosis is expected to be observed in PiZ mice with an average age over 18-month or more, currently young mice (started at age 3w) are being treated with both compound 1 and compound 3.

Follow On Funding: University of Florida - $5,000

Collaborations: This study is a collaboration with Dr. Robert W Huigens III, College of Pharmacy, University of Florida; Dr. Mark Brantly, Devision of Pulmonary, Critical Care and Sleep Medicine, College of Medicine, University of Florida; and Dr. Marc Giulianotti, Torry Pines Institute for Molecular Studies.

Journals: None at the time of reporting.

Patents: Chen Liu, David A Ostrov, University of Florida. Compounds and methods for treatment of Alpha-1 Antitrypsin deficiency. Publication number WO2008143633 A3. Application number PCT/US2007/022717.

6. **Grant #5JK06:** Proliferative Signatures to Predict Benefit of Adjuvant Chemotherapy in Early Stage Non-Small Cell Lung Cancer

   Principal Investigator: William D. Cress, PhD

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: The original purpose of this grant was to invent a laboratory test that could distinguish which early-stage lung cancer patients would benefit from post-surgery chemotherapy from those who would not benefit. This test would spare patients with low test scores from needlessly receiving chemotherapy and experiencing its toxicity. During the first two years of funding, the researchers were able use NanoString-based molecular assays to accurately predict which lung adenocarcinoma patients were likely to benefit from chemotherapy. However, during those two years it became apparent that oncologists were becoming much more interested in finding markers for immunotherapy, since immunotherapy was giving much better results (up to 1/5 of patients receiving long-term benefit).

   In the past year, the research staff have used the vast amount of molecular data and samples collected during the first two years of this project to address molecular markers for immunotherapy and have expanded the approach to include an extensive search for metabolic markers. It has been discovered that about 1/3 of non-small cell lung cancers produce high levels of putrescine (a compound named for it putrid smell) because of a mutation in the gene for Serine/Threonine Kinase 11 (STK11). This putrescine drives away T cells that would otherwise kill the tumor cell. These results not only explain why these tumors escape the immune system and immunotherapy, but also provide a solution since the production of the putrescine is dependent upon an enzyme, ornithine decarboxylase that can be inhibited by an FDA-approval drug, difluoromethylornithine.

   In the final months of this grant, the research team will be writing up their data for publication as well as submit applications for follow up funding. Two types of grants might be plausible. One will be based on basic science and will explore how tumor with different mutations evade the immune system. Also proposed is a clinical trial in which patients with STK11 mutation (high putrescine) will be treated with a compound that will block the synthesis of putrescine. This treatment could increase the fraction of patients that benefit from immunotherapy.

   Follow On Funding: National Institute of Health/National Cancer Institute - $175,312 and $1,250,000

   Collaborations: This project is in collaboration with the University of South Florida, Cancer Biology Ph.D. Program, Nicholas Gimbrone; Undergraduate program, Trent Percy.

   Journals: Chen, L., Kurtyka, C.A., Welsh, E.A., Rivera, J.I., Engel, B.E., Muñoz-Antonia, T., Yoder, S.J., Eschrich, S.A., Creelan, B.C., Chiappori, A.A., Gray, J.E., Ramirez, J.L., Rosell, R., Schabath, M.B., Haura, E.B., Chen, D-T., and Cress, D.W. (2016). Early 2 factor (E2F) deregulation is a prognostic and predictive biomarker in lung adenocarcinoma. Oncotarget. 7(50): 82254-82265 doi:10.18632/oncotarget.12672

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NanoString-based assay to score STK11 (LKB1) pathway disruption in lung adenocarcinoma. J. Thorac. Oncol. 11(6): 838-849 doi:10.1016/j.jtho.2016.02.009

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Teer, J. K., Zhang, Y., Chen, L., Welsh, E. A., Cress, W. D., Eschrich, S. A., & Berglund, A. E. (2017). Evaluating somatic tumor mutation detection without matched normal samples. Human Genomics. 11:22. doi:10.1186/s40246-017-0118-2

Patents: None at the time of reporting.

APPENDIX K

FISCAL YEAR 2017-2018 COMPLETED GRANTS,
Funding Fiscal Year 2016-2017

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
7JK06	Florida A&M University	Sachdeva, Mandip S.	$ 94,810	$ 94,601.03	$ 208.97	3/27/2017	3/31/2018	No	No	No

COMPLETED GRANTS FISCAL YEAR 2017-2018
(Funding Year 2016-2017)

1. **Grant #7JK06:** Oral Nanotechnology in Triple Negative Breast Cancer

   Principal Investigator: Mandip S. Sachdeva, PhD

   Organization: Florida A&M University

   Progress Report: The principle investigator (PI) made a formulation of Piperlongumine and Docetaxel which was evaluated in vitro against Triple negative breast tumors cells and also normal epithelial cells and was found to be more toxic against tumor cells than normal cells. This suggests that toxicity with this formulation will be minimal compared to Docetaxel presently being given parenterally.

   Pharmacokinetic studies in mice also showed that the half-life of Docetaxel was increased when used in combination with Piperlongumine suggesting that Docetaxel can be given orally. These results suggest that this combination could sustain significant levels of the anticancer agent Docetaxel in the blood when given orally. Docetaxel is normally given intravenously with an injectable formulation. Hence there is significant translational application of this research. This will significantly impact many Floridians and also US nationals in having a pill form of Docetaxel which is now given parenterally. This also suggests that breast cancer patients would not have to go to hospitals for chemotherapy and can have this medication in their homes.

   Follow On Funding: None at the time of reporting.

   Collaborations: The research team has been collaborating with Dr. Stephen Safe, Department of Veterinary Physiology & Pharmacology, Texas A&M University and Dr. Arun Kumar Rishi, Department of Oncology, Wayne State University, for work related to this project.

   Journals: None at the time of reporting.

   Patents: None at the time of reporting.

APPENDIX L

FISCAL YEAR 2017-2018 COMPLETED GRANTS,
Funding Fiscal Year 2013-2014

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
4KB16	University of Florida	Shenkman, Elizabeth	$ 1,600,000	$ 1,481,934.74	$ 118,065.26	6/26/2014	12/31/2017	No	Yes	Yes
4KB17	H. Lee Moffitt Cancer Center	Antonia, Scott	$ 1,600,000	$ 1,572,919.36	$ 27,080.64	6/26/2014	12/31/2017	No	Yes	Yes

COMPLETED GRANTS FISCAL YEAR 2017-2018
(Funding Year 2013-2014)

1. **Grant #4KB16:** OneFlorida Cancer Control Alliance

   Principal Investigator: Elizabeth A. Shenkman, PhD

   Organization: University of Florida

   Progress Report: Research Network Development—The OneFlorida administrative team on-boarded three tobacco-related projects in the consortium. OneFlorida has 38 funded projects and 91 active projects operating in the consortium. Of the 78 active projects, 35 focus on cancer and cardiovascular disease. Data Trust Program Core Limited provides an important data set infrastructure for conducting studies focused on tobacco-related cardiovascular disease and cancer. Community Engagement Program Core, a citizen scientist curriculum, is in the final stages of editing and consortium staff will use the curriculum as a training module for on-boarding new citizen scientists.

   To date, the Consent2Share implementation has successfully enrolled over 32,076 adult patients. The signed patient consent and authorization allows investigators to review these patients' EHRs. In addition, the OneFlorida IRB can allow investigators to contact potentially qualified patients who otherwise would have been unknown and unavailable to the researchers.

   OneFlorida Cancer Control Alliance (CCA) has developed an Executive Committee to include representation across the state of Florida and created a citizen scientist training to enable community members to contribute to scientific development. In addition, the OneFlorida CCA has 11 statewide partners that have joined the local centralized IRB and the OneFlorida Data Trust. The OneFlorida CCA sent a finder to the Florida Cancer Data System to link its data to electronic health record data housed in the OneFlorida Data Trust. This linkage will create data profiles for patients in Florida. The OneFlorida community members developed an online, self-paced curriculum to train other laypersons using a web-based platform. The curriculum contains modules on research ethics, sponsored research, clinical and translational science, stakeholder engagement, cultural diversity in research, and biomedical informatics.

   Follow On Funding: Patient-Centered Outcomes Research Institute - $8,089,206.63; National Cancer Institute - $371,547; Agency for Healthcare Research and Quality - $1,344,184

   Collaborations: This project was working with the University of Miami; Florida State University; Florida Agricultural and Mechanical University; Edward Waters College; and Bethune-Cookman University.

   Journals: Hicks, A., Hanna, J., Welch, D., Brochhausen, M., Hogan, W.R. (2016). The ontology of medically related social entities (OMRSE): recent developments. Journal of Biomedical Semantics. doi:10.1186/s13326

   Salloum, R.G., Getz, K.R., Tan, A. S.L., Carter-Harris, L., Young-Wolff, K.C., George,T.J. Jr., Shenkman, E.A. (2016). Use of Electronic Cigarettes Among Cancer Survivors in theU.S. American J. of Preventive Medicine. Nov. doi: 10.1016/j.amepre.2016.04.015

   Salloum, R.G., Thrasher, J. F., Getz, K.R., Barnett, T.E., Asfar, T., Maziak, W. Patterns of Waterpipe Tobacco Smoking Among U.S. Young Adults, 2013−2014. American J. of Preventive

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Yuan, J., Malin, B., Modave, F., et al. (2017). Towards a privacy preserving cohort discovery framework for clinical research networks. Journal of Biomedical Informatics. 66:42-51. doi:10.1016/j.jbi.2016.12.008

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Yang, C., Delcher, C., Shenkman, E.A, Ranka, S. (2017). Machine learning approaches for predicting high utilizers in healthcare. Bioinformatics and Biomedical Engineering. IWBBIO. LNCS. 10209:e382-395. doi: 10.1007/978-3-319-56154-7_35

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Patents: None at the time of reporting.

2. **Grant #4KB17:** Expansion of Enduring Infrastructure to Support Lung Cancer Screening Research

   Principal Investigator: Scott Antonia, MD, PhD

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: The goals of this project were to expand and improve existing infrastructure to support lung cancer screening research at the Moffitt Cancer Center. The objectives were the following, to: 1) address barriers to lung cancer screening, 2) establish lung cancer screening registry, 3) collect and process advanced digital features from computerized tomography (CT) scans, and 4) develop a smoking cessation program specifically for screening participants. Findings have been published from focus groups with health care providers and high-risk individuals. The research team has implemented a system which allows for the development of database containing risk factors and outcomes among screening participants. A uniform reporting format based on a Lung CT Screening Reporting & Data System (Lung-RADS) has been adopted. Structured interviews among primary care physicians and high-risk participants have been conducted to help address barriers to lung cancer screening. Based on the four objectives within this study, the researchers have acknowledged the following results.

   Aim 1: The team has worked with the Public Relations and Marketing department at Moffitt Cancer Center to build a web-based platform where training materials and study findings are housed. The portal is readily accessible to providers via this link https://moffitt.org/for-healthcare-providers/clinical-programs-and-services/thoracic-oncology-program/treatmentsservices/lung-cancer-screening-surveillance/lung-screening-research-and-resources/. Additionally, providers

are able to directly refer patients for low-dose CT (LDCT) screenings using this portal. Two manuscripts were submitted in 2016 and both were accepted and published in 2017.

Aim 2: Two new research coordinators were hired to manage recruitment and patient follow-up visits at all Moffitt Cancer Center locations. As of period three, 294 patients were consented. In addition to new marketing efforts, the research team has partnered up with Millennium Physicians group to increment accrual number and expand the study population to included incidental pulmonary nodule patients. They have curated a retrospective cohort of 2600 patients with incidental pulmonary nodules, including images and data, and are prospectively recruiting patients that present with incidental pulmonary nodules. To date, they have prospectively recruited 67 participants. Among the prospective patients, the researchers are collecting the same biospecimens for the incidental pulmonary nodule patients as they do for the lung cancer screening participants. They have also expanded their electronic database to include Lung-RADS (a lung imaging reporting and data system), CT types, and CT results as new variable. As such, new queries were developed for data analyses and reporting. Additionally, Tissue Core's LabVantage software and biosspecimen database were incorporated to the project's electronic dashboard to help track biosamples and simplify collection of processed bio-specimens (DNA, buffy coat and plasma). Research coordinators continued to review and confirm patient eligibility based on National Lung Screening Trial criteria.

Aim 3: The research team has finalized the latest updates of the software to the Quantitative Imaging Decision Support (QIDS ®) platform, which generates pre-processing of LDCT data to enable radiologic consistency and efficiency. These have been used to quantitatively analyzed screening subjects and high-impact manuscripts have either been accepted or are in review.

Aim 4: Recruitment and follow-ups for the pilot smoking cessation randomized clinical trial (RCT) have been completed. Of all the patients undergoing a LDCT lung screen who were screened (n=87), 25% were smokers and met eligibility criteria. Of all eligible (n=22), 82% consented and enrolled. Retention rates were as follows: 1m = 84%, 6m = 68%, and 9m = 58%. Following completion of the pilot RCT, 9 study participants were re-contacted and completed in-depth interviews to further evaluate the new intervention and the participants' experience during and after LDCT screening.

Follow On Funding: National Cancer Institute/National Institute of Health - $3,467,027, $84,250 and $152,906

Collaborations: This research project has collaborated with Vanderbilt University, the University of South Florida, Boston University, and the Millennium Physician Group.

Journals: Hudson, J.N., Quinn, G.P., Wilson, L.E., Simmons, V.N. (2017). Evaluation of promotional materials to promote Low-Dose Computed Tomography (LDCT) screening to high risk consumers and health care providers. J. Canc. Ed. 1-9 doi:10.1007/s13187-017-1204-9

Simmons, V.N., Gray, J.E., Schabath, M.B., Wilson, L.E., Quinn, G.P. (2017). High-risk community and primary care providers knowledge about and barriers to low-dose computed tomography lung cancer screening. Lung Cancer 2017.106:e42-29 doi:10.1016/j.lungcan. 01.012 PMID: 28285693

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Hawkins, S., Wang, H., Liu, Y., Garcia, A., Stringfield, O., Krewer, H., Li, Q., et al. (2016).

Predicting malignant nodules from screening CT Scans. J Thorac. Oncol. 11:12; e2120-2128. doi: 10.1016/j.jtho.2016.07.002

Piñeiro, B., Simmons, V.N., Palmer, A.M., Correa, J.B., Brandon, T.H. (2016). Smoking cessation interventions within the context of Low-Dose Computed Tomography lung cancer screening: A systematic review. J Lung Cancer. 6.98:e91-98. doi:10.1016/j.lungcan.2016.05.028

Patents: None at the time of reporting.

Live Like Bella Pediatric Cancer Research Initiative

APPENDIX M

FISCAL YEAR 2017-2018 NEWLY AWARDED 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	$ 11,111.00	$ 188,889.00	5/11/2018	4/30/2021	No	No	No
8LA02	University of Central Florida	Fernandez-Valle, Cristina	$ 200,000	$ 11,111.00	$ 188,889.00	6/14/2018	4/30/2021	No	No	No
8LA03	University of Miami	Robbins, David J.	$ 200,000	$ 11,111.00	$ 188,889.00	5/21/2018	4/30/2021	No	No	No
8LA04	Baptist Health South Florida	Hall, Matthew	$ 700,000	$ 29,166.66	$ 670,833.34	6/14/2018	4/30/2022	No	No	Yes
8LA05	Florida International University	Azzam, Diana	$ 700,000	$ 29,166.00	$ 670,834.00	5/11/2018	4/30/2022	No	No	No

NEWLY AWARDED GRANTS FISCAL YEAR 2017-2018

(Funding Year 2017-2018)

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

   Abstract of Proposed Research: This research proposes to understand one of the root causes of resistance of childhood acute lymphoid leukemia (ALL). ALL is the most common childhood cancer and while ~80% of children can be cured, a unfortunate fraction of children suffer relapses. While chimeric antigen receptor T cell (CAR-T) therapy may be an option, this is not yet widely available in Florida. There are several mutations associated with early relapse of ALL. Currently, the researchers are studying one of these, an activating mutation of the NSD2 (nuclear receptor binding SET domain protein 2) gene. The NSD2 is a histone methyltransferase that can turn off or suppress the activity of certain genes. When NSD2 mutations occur, it has been shown to activate an abnormal gene expression program in the malignant blood cells. This gene expression program drives aggressive biological behavior of the tumor cells including brain invasion and resistance to therapy. In this past quarter, the researchers examined how the NSD mutation changes the ability of leukemia cells to respond to glucocorticoids (E.g. prednisone, steroids) an anti-inflammatory drug used a main feature of childhood leukemia treatment. The NSD2 mutant cells are resistant to the treatment and fail to activate several specific cell death causing genes. When the NSD2 mutation is removed from the leukemia cell using modern gene editing techniques, the cells once again become sensitive to therapy and the pro-death genes are activated. In the next quarter the researchers will examine at the detailed molecular level why the glucocorticoids fail to active the death genes and determine if there are ways to work around this blockade and re-sensitize the cells to therapy.

   Follow On Funding: None at the time of reporting.

   Collaborations: None at the time of reporting.

   Journals: None at the time of reporting.

   Patents: None at the time of reporting.

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

   Principal Investigator: Cristina Fernandez-Valle, PhD

   Organization: University of Central Florida

   Abstract of Proposed Research: Neurofibromatosis type 2 (NF2) causes tumors to grow on nerves throughout the body (schwannomas) and in the brain (meningiomas and ependymomas). NF2 affects around 1 in 25,000 individuals worldwide. NF2 is caused by mutations in the Neurofibromatosis Type 2 gene that encodes a tumor suppressor called merlin. A diagnostic criterion for this disorder is the development of schwannomas on both hearing and balance nerves; these tumors are called vestibular schwannomas or acoustic neuromas. NF2 patients develop multiple schwannomas on other nerves, and multiple brain and spinal tumors such as

meningiomas and ependymomas. Most patients develop symptoms in their teenage years or during early childhood. Tumor control early in life is critical for maintaining a quality of life and preventing malignancies. Currently, potential treatments available for NF2-associated tumors are surgery, chemotherapy, and radiation therapy. However, due to location some tumors are inoperable and there are no Food and Drug Administration (FDA) approved drug therapies that shrink or stop the growth of schwannoma tumors. Undoubtedly, there is a great need for pharmaceuticals to prevent tumors from growing and shrinking or slowing the growth of existent tumors. The development of NF2 drug therapies has been challenged by lack of relevant merlin-deficient Schwann cell lines, animal models and clear druggable target because merlin lacks enzymatic activity. To address these deficits, the research lab has created a panel of human and mouse cell models of the disease, developed high-throughput viability and high- content multi-parametric assays, conducted multiple screening campaigns of compound libraries using their Schwann cell lines, and optimized a sciatic nerve allograft mouse model in immune-deficient mice. The researchers have performed an unbiased chemical genomics approach and identified several phosphoinositide 3-kinase (PI3K) and PI3K/mTOR inhibitors that selectively reduce viability NF2 model cells compared to control cells.

However, kinome analysis of NF2 model cells chronically exposed to PI3K inhibitors revealed that cells compensated by consistently increasing activity of focal adhesion kinase (FAK) and SRC (proto-oncogene tyrosine-protein kinase) family members. Rewiring of kinase networks occurs in cancer cells developing drug resistance to monotherapies. Moreover, an exploratory combination drug screen identified the PI3K inhibitor GSK- 2126458 (omipalisib) and the FAK inhibitor, TAE226, as well as other pathway inhibitors synergistic and selective for merlin deficient Schwann cells. These results suggest that targeting compensatory pathways in combination with PI3K inhibitors should provide sustained inhibition of merlin-deficient Schwann cell proliferation and/or survival. To advance these findings, the researchers propose to screen the effectiveness of PI3K and secondary compensatory (alone and in combination) in multiple mouse and human merlin-deficient Schwann cell lines, and to study in vivo the best synergic combination (individual and combined drugs), using their orthotopic allograft model of NF2 schwannomas. The researchers expect to obtain the necessary preclinical data to support their potential use in NF2. To optimize translation into clinical trials, the in vivo preclinical study will be designed and conducted in consultations with their collaborators, Drs. Smith and Aguilar- Bonilla, the pediatric neuro-oncologists at the Arnold Palmer Hospital for Children in Orlando.

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 #8LA03:** Novel Regulators of SHH-Driven Medulloblastoma

   Principal Investigator: David J. Robbins, PhD

   Organization: University of Miami

   Abstract of Proposed Research: Tumor recurrence observed in medulloblastoma (MB) patients treated with the Sonic Hedgehog (Shh) inhibitor vismodegib12 highlights the need for alternative targeted therapies to treat these pediatric tumors. Consistent with this goal they provide preliminary data showing that the covalent modification (methylation) of Gli proteins can modulate

their activity or stability. They hypothesized that the identification and targeting of these Gli methylases will attenuate the growth of pediatric Shh driven MB. The researchers proposed to: Aim 1. Elucidate the role Gli methylation plays in regulating its function. Their preliminary data suggests that Gli protein activity is regulated by methylation of a subset of distinct arginine residues. They proposed to identify the methylation sites on Gli1 and Gli2 relevant in MB. They would then engineer Gli mutants that lack, or mimic, different combinations of these methylation sites, and determine the effects of methylation on Gli activity, stability or localization. They will subsequently test the functional role Gli methylation plays in MB growth using MB sphere cultures that are dependent on Shh activity13. The research team will also identify the methyltransferases (PRMT) responsible for Gli methylation in vivo14. Aim 2. Determine the role their candidate Gli methylases play in modulating MB growth in vivo. They will test the hypothesis that the Gli methylases they have identified will play pivotal roles in MB development, using both mouse and patient derived Shh-subgroup MB mouse models. To achieve this goal, the team will implant mouse MB sphere cultures orthotopically into mice, modulate the expression of the most relevant PRMT candidates from Aim 1 using an inducible short hairpin-RNA system, and assess changes in tumor growth in vivo. Additionally, patient derived xenograft MB models will be treated with small molecule inhibitors that target these relevant methylases, focusing on those currently undergoing clinical evaluation, and MB growth evaluated.

The work proposed herein will address the substantial clinical need for novel druggable targets in Shh-driven cancers, especially for those patients with recurrent, vismodegib resistant tumors.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Organization: Miami Cancer Institute, Baptist Health South Florida

Abstract of Proposed Research: Brain tumors are the most common primary solid tumor in children in the United States. Although >75% of children with brain tumors are cured, survivors are at considerable risk for developing late toxicities. Radiotherapy (RT) is a critical component of treatment for many children with brain tumors, but can cause significant morbidity, adversely affect quality of life, and limit school and work success in later life. Dosimetric studies have shown the risk of endocrine and neurocognitive toxicities following radiation to specific brain regions. To date, however, the effects of brain radiation have been quantified in few brain substructures.

The research project proposes a novel multi-institutional clinical trial to measure changes in brain substructure anatomy and volumes in children following radiation to the brain. Exposure of brain substructures to radiation will be measured prospectively and changes in these substructures will be measured over time. The primary goal of this project is to model the dose-volume relationship for brain changes in response to radiation and to correlate these changes with the development of neurocognitive, endocrine, and quality of life effects. All children who receive conventional or

proton radiation to the brain will be eligible to enroll in this research trial. MRI will be performed before and after radiation. Anatomic changes in the brain will be measured using the NeuroQuant software package (CorTechs Labs, San Diego, CA). This validated program analyzes MRIs and identifies and measures >50 anatomical brain structures. Changes in these brain structures can be measured over time and compared to normal patients of the same age and gender. Neurocognitive testing, endocrine status, and health-related quality of life (HR-QOL) will be measured at baseline and at pre-specified time points during the follow-up period.

The primary goal of this project is to measure the magnitude of changes in the brain after exposure to radiation over time and establish a dose-effect relationship. They hypothesize that measurable changes will occur in the brain following exposed to high, low, and even no radiation dose. The secondary objective is to correlate the magnitude of the observed changes with the development of measurable late effects on neurocognitive function, hormonal deficiencies, and changes in HR-QOL.

In this protocol, the research team will also measure cytokine levels and molecular biomarkers in the blood at baseline and after radiation in an exploratory manner and seek to identify potential associations with the risk of developing toxicities due to radiation. Blood will be collected from consenting patients before and after radiation. Levels for pro-inflammatory and anti-inflammatory cytokines (IL-1, IL-2, IL-4, IL-6, IL-10, TNFα) will be measured. In addition, Apolipoprotein E, Catechol-O-methyltransferase (COMT), and brain-derived neurotrophic factor (BDNF) genotype polymorphisms, which have been implicated in “Chemo Brain”, will also be assessed. They seek to determine if these cytokine levels and genetic polymorphisms are associated with late toxicities.

With a better understanding of how radiation affects the brain, clinicians may better counsel families regarding the risks of radiation and develop strategies to minimize them. This will potentially result in major reductions in toxicities in childhood cancer survivors.

Follow On Funding: An Institutional Foundation Grant - $100,000

Collaborations: This project is working with the University of South Florida and Nicklaus Children’s Hospital in the Department of Neurosurgery and Pediatric Neuropsychology.

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

Abstract of Proposed Research: The major challenge in the clinical management of pediatric cancers is the development of effective and targeted therapies for the 30-40% of children with certain types of leukemia and solid tumors, in whom no remission occurs or who suffer relapse. Children with recurrent and refractory cancers often have very limited standard therapy options and are suffering from various treatment-related complications. Despite increased efforts in whole genome screening of pediatric cancers, much work remains to be done in characterizing germline and somatic mutations with matching drugs and appropriate targeted treatment. They have previously implemented and validated a combined functional drug screening and genomics

platform to tailor treatments to individual patients with adult refractory cancers. This personalized approach enabled clinical application of individualized treatment for refractory patients with no alternative options. Here, they intend to adopt this approach in refractory pediatric cancers to guide clinical decision making and provide novel therapeutic options for children, selecting drugs from a library of the Food and Drug Administration (FDA)-approved oncology drugs to offer patients the safest possible chance of achieving remission and ultimately cure of their disease. In this pilot feasibility study, they intend to enroll chemorefractory or relapsed patients with all types of cancers where tumor tissue would be available for drug screening and genomic profiling. Using their robust high-throughput ex vivo drug sensitivity assay and combining it with mutation analysis, they will be able to create a compendium of drug responses in individual patients, match actionable mutations with selective targeted therapies and clinically apply individual treatment for refractory patients with no alternative options.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Zika Research Grant Initiative

APPENDIX N

FISCAL YEAR 2017-2018 COMPLETED GRANTS,
Funding Fiscal Year 2016-2017

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditures	Unspent	Executed Date	End Date	Patents	Publications	Follow-on Funding
7ZK01	University of Miami	Daunert, Sylvia	$ 1,141,585	$ 526,884.00	$ 614,701.00	3/01/2017	6/30/2018	No	No	No
7ZK02	Florida State University	Holmes, Eric	$ 1,113,645	$ 513,990.00	$ 599,655.00	3/09/2017	6/30/2018	No	No	No
7ZK03	University of Miami	Strbo, Natasa	$ 981,901	$ 453,186.00	$ 528,715.00	3/01/2017	6/30/2018	Yes	No	No
7ZK04	University of Central Florida	Huo, Qun	$ 199,280	$ 194,473.94	$ 4,806.06	3/08/2017	6/30/2018	Yes	Yes	No
7ZK05	University of Central Florida	Chumbimuni - Torres, Karin	$ 198,875	$ 191,221.40	$ 7,653.60	3/08/2017	6/30/2018	No	Yes	No
7ZK06	Florida State University	Megraw, Tim	$ 856,750	$ 388,158.82	$ 468,591.18	3/08/2017	6/30/2018	No	Yes	No
7ZK07	The Scripps Research Institute	Choe, Hyeryun	$ 199,280	$ 199,280.00	$ 0.00	3/08/2017	3/31/2018	No	No	No
7ZK08	University of Miami	Bandstra, Emmalee S.	$ 1,989,654	$ 913,448.41	$ 1,076,205.59	3/09/2017	6/30/2018	No	Yes	Yes
7ZK09	Florida International University	El-Hage, Nazira	$ 1,984,536	$ 841,134.24	$ 1,143,401.76	3/09/2017	6/30/2018	No	Yes	No
7ZK10	Florida Atlantic University	Asghar, Waseem	$ 199,280	$ 199,280.00	$ 0.00	3/08/2017	6/30/2018	No	Yes	No
7ZK11	University of Miami	Deo, Sapna K.	$ 199,280	$ 189,351.23	$ 9,928.77	3/08/2017	3/31/2018	No	No	No
7ZK12	University of Florida	Nguyen, Cuong	$ 868,744	$ 399,183.19	$ 469,560.81	3/08/2017	6/30/2018	No	Yes	Yes
7ZK13	Nova Southeastern University	Beljanski, Vladimir	$ 198,886	$ 195,407.49	$ 3,478.51	3/08/2017	6/30/2018	No	No	No

7ZK14	University of Miami	Saigal, Gaurav	$ 1,141,457	$ 439,020.00	$ 702,437.00	3/09/2017	6/30/2018	No	Yes	Yes
7ZK15	University of Florida	Alto, Barry W.	$ 199,144	$ 197,050.40	$ 2,093.60	3/14/2017	3/31/2018	No	Yes	No
7ZK16	Florida State University	Meckes, David G.	$ 199,280	$ 189,232.33	$ 10,047.67	3/01/2017	6/30/2018	No	Yes	No
7ZK17	University of Central Florida	Parks, Griffith D.	$ 500,408	$ 318,706.81	$ 181,701.19	3/01/2017	6/30/2018	No	No	No
7ZK18	University of Florida	Morris Jr., John G.	$ 198,812	$ 191,938.34	$ 6,873.66	3/01/2017	6/30/2018	No	No	No
7ZK19	University of South Florida	Casale, Thomas B.	$ 1,117,413	$ 429,775.00	$ 687,638.00	3/02/2017	6/30/2018	No	Yes	No
7ZK20	University of Miami	Younis, Ramzi T.	$ 1,140,125	$ 437,390.00	$ 702,735.00	3/09/2017	6/30/2018	No	Yes	Yes
7ZK21	University of Miami	Barber, Glen N.	$ 1,141,582	$ 508,691.85	$ 632,890.15	3/09/2017	6/30/2018	Yes	Yes	No
7ZK22	University of Florida	Fan, Hugh Z.	$ 515,377	$ 230,759.75	$ 284,617.25	3/01/2017	6/30/2018	Yes	Yes	No
7ZK23	University of South Florida	Lockwood, Charles J.	$ 1,141,582	$ 526,884.00	$ 614,698.00	3/07/2017	6/30/2018	No	Yes	No
7ZK24	University of Miami	Martinez, Claudia	$ 963,109	$ 370,426.50	$ 592,682.50	3/08/2017	6/30/2018	No	No	No
7ZK25	Florida International University	DeGennaro, Matthew J.	$ 198,468	$ 198,468.00	$ 0.00	3/08/2017	3/31/2018	No	No	No
7ZK26	University of Miami	Gonzalez, Ivan A.	$ 1,989,654	$ 765,251.50	$ 1,224,402.50	3/09/2017	6/30/2018	No	Yes	Yes
7ZK27	University of Miami	Stevenson, Mario	$ 1,141,582	$ 445,694.81	$ 695,887.19	3/07/2017	6/30/2018	No	No	No
7ZK28	University of Miami	Dhar, Shanta	$ 1,141,582	$ 505,315.00	$ 636,267.00	3/07/2017	6/30/2018	No	No	No
7ZK29	H. Lee Moffitt Cancer Center	Monteiro, NA Alvaro	$ 199,280	$ 195,128.58	$ 4,151.42	3/08/2017	3/31/2018	No	No	No
7ZK30	University of Florida	Brown, Ashley N.	$ 1,140,922	$ 465,799.39	$ 675,122.61	3/07/2017	6/30/2018	No	Yes	No
7ZK31	University of Miami	Sharkey, Mark E.	$ 199,273	$ 199,273.00	$ 0.00	3/08/2017	3/31/2018	No	No	No
7ZK32	University of Central Florida	Bagci, Ulas	$ 199,254	$ 159,404.00	$ 39,850.00	3/09/2017	3/31/2018	No	No	No
7ZK33	University of Central Florida	Gerasimova, Yulia	$ 200,000	$ 197,242.52	$ 2,757.48	3/08/2017	6/30/2018	No	No	No
7ZK34	University of South Florida	Teng, Michael N.	$ 200,000	$ 198,806.29	$ 1,193.71	2/28/2017	6/30/2018	No	Yes	No

COMPLETED GRANTS FISCAL YEAR 2017-2018
(Funding Year 2016-2017)

1. **Grant #7ZK01:** Antibody Based Zika Diagnostics

   Principal Investigator: Sylvia Daunert, PhD

   Organization: University of Miami

   Progress Report: In 2016 Miami emerged as the US epicenter of the then-global Zika virus (ZIKV) epidemic. The consequences of infection remain to be fully elucidated, but the probable link between ZIKV infection and fetal developmental complications raised enormous concern as to the potential impact of ZIKV. The urgent needs were to identify individuals at risk for ZIKV infection (pregnant women and couples wishing to become pregnant). The goals of this project were to 1) develop diagnostics for the detection of the virus in the acute phase and 2) to develop an assay for previous exposure to ZIKV. Dr. Daunert and associates successfully refined and tested their ZIKV diagnostic assays for both the presence of virus in the acute phase and serological activity in the convalescent phase. They developed a rapid, sensitive, and accurate immunoassay for the detection of ZIKV in human physiological fluids including urine and 10-fold dilutions of saliva, serum, and whole blood. The calculated detection limit for each respective physiological fluid fell within the range of reported ZIKV levels. The team successfully demonstrated that their monoclonal antibodies (mAbs) bind specifically to ZIKV and do not bind to any of the four serotypes of Dengue Virus (DENV).

   They simultaneously used their anti-ZIKV mAbs in a serum competition assay to determine previous exposure to ZIKV. Dr. Daunert's team tested their convalescent phase assay against a number of patient samples, co-circulating pathogens, interfering substances, and naive human samples. Additionally, they translated their laboratory based diagnostic assay into a preliminary point of care (PoC) lateral flow assay (LFA) for rapid detection of ZIKV infection that can be carried out in the doctor's office in 30 minutes or less.

   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 #7ZK02:** Human Pharmacokinetics of Niclosamide

   Principal Investigator: Eric H. Holmes, PhD

   Organization: Florida State University

   Progress Report: The purpose of this project was to explore the potential of using an existing Food and Drug Administration (FDA) approved drug, Niclocide, for general treatment of Zika virus infections in the population. Niclosamide, the active ingredient in Niclocide, an FDA approved drug for tape worm infections, has been shown to have an infectious dose-50 of 0.22 uM for inhibition of Zika virus replication. Rat studies have shown that an oral dose of 5 mg/Kg of niclosamide yields a blood level of 1.08 uM. The approved dose of Niclocide is 2 grams/day, or about 25- to 33 mg/Kg body weight. Pharmacokinetic (PK) studies had not yet been conducted on humans and the intent

was to determine if the approved 2 gram/day dose would be adequate to achieve a blood level in the 3- to 10 micromolar range. Given the significant public health threat posed by Zika, especially in pregnant women, the availability of an easily adapted marketed drug for Zika therapeutics represented a major opportunity. In particular, use of an off-the-shelf therapeutic could have had a rapid and critical impact. In aim 1, Dr. Holmes and his team developed and conducted a human pharmacokinetic analysis of single and multiple doses of 2 grams of Niclocide. A fed/fasted PK analysis was conducted on 12-15 normal individuals in a cross-over study. These individuals were given 3 doses of Niclocide at 24 hour intervals. Blood levels of niclosamide were determined at specified time points and PK parameters were determined based on the data collected. In aim 2, they performed a single group multiple dose case-control study, with a 7-day administration of Niclocide daily to newly diagnosed Zika infected subjects versus a placebo control group. A group of 6 newly diagnosed Zika-infected individuals were randomized (4 test, 2 placebo) and treated with either Niclocide or a placebo daily for 7 days. Virus titers in the blood and symptom scores were determined daily. The results from this clinical test were compiled and submitted to a journal for publication.

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 #7ZK03:** Development and Testing of Novel Secreted GP96-Ig Zika Virus (ZIKV) Vaccine

   Principal Investigator: Natasa Strbo, MD, PhD

   Organization: University of Miami

   Progress Report: Targeting the ZIKV infection with vaccines offered the best opportunity to generate protective immunity in most vulnerable population: child-bearing women, before virus had a chance to cross placental barrier and infection takes hold in fetus. The vaccine approach in this study was focused on generating a high magnitude of placental ZIKV-antigen specific Cytotoxic T cells (CD8+ CTL) and Th1 type antibody responses and while also evaluating the vaccine immunogenicity and efficacy in pregnant mouse model. Dr. Strbo and associates successfully generated a vaccine vector and cells expressing secreted gp96-Ig and ZIKA antigens as proposed in their Aim 1. Completion of the generation of gp96-Ig-ZIKA vaccine cell served as an initial “go", “no-go” milestone for this study and they successfully reach this milestone within the first 6 months of the grant period. In addition, the team completed most of the immunogenicity experiments in the mouse pregnancy model testing different vaccine platforms: cell based and DNA based secreted gp96-Ig vaccine approach. Their findings were highly supportive of further development of novel gp96-Ig vaccine as unique placenta-homing, antigen-specific CD8 CTL vaccine strategy. They had an excellent team of researchers working on this project as well as full administrative support of University of Miami Research Office and Department of Microbiology and Immunology. The ZIKA research symposium held in October of 2017 provided a great opportunity to meet other ZIKA researchers and establish collaborations with researchers from other institutions.

   Follow On Funding: None at the time of reporting.

   Collaborations: None at the time of reporting.

   Journals: None at the time of reporting.

Patents: UMIP-114 Invention Disclosure under the Title" Vectors and Vaccine cells for immunity against ZIKA virus" was filed for patent October 10, 2017.

4. **Grant #7ZK04:** Point of Care Assay Development for Diagnosis of Zika Viremia

   Principal Investigator: Qun Huo, PhD

   Organization: University of Central Florida

   Progress Report: In this project, Dr. Huo and team developed and evaluated a rapid point-of-care, field deployable blood test (D2Dx) for Zika virus (ZIKV) infection detection and diagnosis. The new blood test was built upon an innovative, point-of-care diagnostic device platform invented at University of Central Florida, licensed and commercialized by a Florida startup company, Nano Discovery Inc. (Orlando, FL). The new blood test requires only a few drops of blood that can be obtained through finger prick, and it takes approximately 30 min from collecting the blood sample to obtaining the test result. Throughout the life of their project, the team tested and evaluated a large number of ZIKV-infected patient samples, and various control samples. Evaluations study suggested that their new blood test can detect ZIKV infection as early as on day 2 following the onset of symptoms. This diagnostic sensitivity represents a significant improvement over the current serology testing, which could only detect acute ZIKV infection 5-7 days later following the start of the symptoms. Furthermore, their extensive studies conducted on both animal models and human blood samples demonstrated that the new blood test can determine the active status and duration of the infection. This diagnostic capability provides critical clinical information on when a Zika-infected woman is completely cleared of the virus from natural recovery or medical treatment, and thus when pregnancy no longer carries increased risk.

   Follow On Funding: None at the time of reporting.

   Collaborations: The following were involved in this project: The University of Central Florida **(**Dr. Karl McKinstry and Dr. Tara Strutt); four students: Tianyu Zheng (Ph.D.); Christopher J. Parrett (Ph.D.); Kunal Dhume (Ph.D.), and Yuen Yee Li Sip (Undergraduate Student); Colorado State University (Dr. Richard Bowen); University of Miami (Dr. Mario Stevenson); and McGill University (Dr. Martin Richer).

   Journals: Zheng, T., Finn, C., Parrett, C.J., Dhume, K., Hwang, J., Sidhom, D., Strutt, T.M., Sip, Y.Y.L., McKinstry, K.K., Huo, Q. A Rapid Blood Test to Determine the Active Status and Duration of Acute Viral Infection. ACS Infectious Diseases 2017 3 (11), 866-873 DOI: 10.1021/acsinfecdis.7b00137

   Patents: US 62/644,467, "Detection of interaction between an assay substance and blood or blood components for immune status evaluation and immune-related diseases detection and diagnosis", Huo, Q.; Zheng, T.; McKinstry, K. Provisional patent filed on March 17, 2018.

5. **Grant #7ZK05:** A Universal Nucleic Acid Recognition Platform for Detection of Zika Virus

   Principal Investigator: Karin Y. Chumbimuni-Torres, PhD

   Organization: University of Central Florida

   Progress Report: The goal of this project was to develop a fast, specific and sensitive universal point of serving testing (POST) platform for the rapid detection of the Zika virus (ZIKV). To accomplish this aim, Dr. Chumbimuni-Torres and team used a binary probe that is proven to have high specificity for single-nucleus sequencing (SNS) differentiation in ribonucleic acid

(RNA). RNAs are considered key biomarkers for a variety of viral infections. However, these RNAs form secondary structures, complicating their detection. Although previously established approaches offer high sensitivity and rapid results, they are prone to contamination and frequently produce false-positive results and require technical expertise. The new methodology proposed here (i.e. the novel sensor platform) was successfully developed by the researchers, exceeds the performance of current state-of-the-art approaches for RNA sensing in the following aspects: 1) it exhibits zero false-positive responses, thus improving sensitivity and limit of detection (LOD); 2) it enabled accurate recognition of SNS at ambient temperatures in RNA; and 3) it allowed for the detection of multiple analytes using a single universal probe that is reusable. This universal platform for the detection of virus Zika, can complete an analysis in 10 minutes. In addition, their biosensor is capable of detecting Dengue Virus, while using the same probe. The use of a universal probe in this biosensor will facilitate future manufacture. The research team also performed the analysis in amplified NASBA products and differentiated ZIKV from Dengue 1, 2, 3, and 4.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Mills, Dawn M., et al. “A Universal and Label-Free Impedimetric Biosensing Platform for Discrimination of Single Nucleotide Substitutions in Long Nucleic Acid Strands.” *Biosensors and Bioelectronics*, vol. 109, 2018, pp. 35–42., doi:10.1016/j.bios.2018.02.059.

Patents: None at the time of reporting.

6. **Grant #7ZK06:** Mechanism of Centrosome Activation by Zika Virus and the Evaluation of Pharmacological Interventions that Target Centrosome-mediated ZIKV Proliferation

   Principal Investigator: Timothy L. Megraw, PhD

   Organization: Florida State University

   Progress Report: Dr. Megraw’s research lab has made significant progress toward determining how Zika virus (ZIKV) reorganizes the microtubule cytoskeleton to support virus replication in human cells. They discovered that ZIKV activates the centrosomal microtubule organizing center (MTOC) activity, leading to elevated microtubules (MT) assembly at interphase centrosomes in ZIKV infected cells. The levels of several centrosomal proteins are altered, with some proteins being decreased, and one elevated. In addition, they showed that the viroplasm, a subcellular compartment where the virus assembles, is organized in a toroidal structure around the centrosome. Upon treatment of ZIKV-infected cells with Centrinone, an inhibitor of Polo-like Kinase 4 (PLK4) that blocks centrosome duplication, and the subsequent loss of centrosomes, they found that the organization of the viroplasm was compromised. Centrinone (CFI-400945) is currently in Phase 1 clinical trials for treatment of advanced cancer. Thus, they identified Centrinone as a potential drug treatment for ZIKV infection.

   In their more recent investigations, the team discovered that, in addition to the centrosome, that a non-centrosomal microtubule-organizing center at the golgi is also reorganized following ZIKV infection. They observed that elevated MT assembly at centrosomes coincided with organization of MTs at the outer membrane of the viroplasm also. Probing the source of these MTs further, they discovered that the golgi apparatus is reorganized in ZIKV-infected cells such that it becomes intimately associated with the outer membrane of the viroplasm. They will continue their investigations to determine whether the golgi MTOC is essential for ZIKV replication and whether

it functions synergistically with the centrosomal MTOC to support ZIKV replication.

Altogether, these findings significantly advanced their understanding of the regulation of host cell MTOCs by ZIKV, and how these reorganized MTOCs support ZIKV proliferation. Their work is expected to have a significant impact on the field and with a publication to come in the next few months. Their project benefited immensely from their collaboration with Hengli Tang at FSU, an expert in Flaviviruses. Unfortunately, their progress on this project and the support their grant provided to employees and trainees on the project were negatively impacted by the sudden cancellation of the ZIKV funding.

Follow On Funding: None at the time of reporting.

Collaborations: This project was working with the Department of Biological Sciences at Florida State University (Dr. Hengli Tang, five students, two Ph. D students); and Dr. Megraw's section (two PhD students and one undergraduate student).

Journals: Tillery, M.M.L., Blake-Hedges, C., Zheng, Y., Buchwalter, R.A., Megraw, T.L. Centrosomal and Non-Centrosomal Microtubule-Organizing Centers (MTOCs) in Drosophila melanogaster. Cells. 2018; 7(9):121.

Patents: None at the time of reporting.

7. **Grant #7ZK07:** Development of High Throughput Screening Tools to Search for Compounds inhibiting the Essential Zika Virus NS3 Protease

   Principal Investigator: Hyeryun Choe, PhD

   Organization: The Scripps Research Institute

   Progress Report: Zika virus (ZIKV) produces a single, large protein molecule during infection that is cleaved by a combination of viral and host proteases to produce the mature viral proteins. The major protease for ZIKV is the novel serine virus protease termed NS2B-NS3, which is essential for ZIKV replication. The goal of this grant was to develop novel inhibitors of the protease necessary for replication of the ZIKV. To do so, Dr. Choe's research team developed a cell-based reporter system that signals when the protease is active. As they planned, during the first quarter, they developed an assay, in which active Zika virus protease produces luminescence. In the second quarter, they optimized this assay for a 96-well format, and then for a 384-well format. In the third funding period, they further optimized the assay to fit a larger-well format and performed a test screen of a small-scale compound set. During the final - current - quarter, they were able to optimize the assay to fit a 1,536-well format, repeated the screen of the small compound library, and obtained similar results as before. Unfortunately, of 19 hits, only two were non- or minimally toxic to cells, and neither of these two were able to inhibit NS2B/3 protease, judging from the results of replicon assays. Therefore, the researchers concluded that those three compounds inhibited other steps of Zika virus replication cycle. With this high-throughput screen assay set up, however, they plan to screen a larger-size library when funds become available.

   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 #7ZK08:** A Prospective, Longitudinal Assessment of Infants of Mothers with Zika Infection in Pregnancy

   Principal Investigator: Emmalee S. Bandstra, MD

   Organization: University of Miami

   Progress Report: This project sought to identify and assess two prospective cohorts of infants from birth through age 24 months to determine effects of maternal Zika infection on infant-toddler outcomes, including neurobehavior (NICU Network Neurobehavioral Scale; NNNS), neurodevelopment (Bayley-lll Scales) and ophthalmologic abnormalities (dilated eye exams with retinal photography). It was anticipated that 150 infants born to mothers with Zika infection would be enrolled for comparison with matched Zika-negative controls. Mother-infant dyads were recruited from Jackson Memorial Hospital (JMH) and University of Miami Miller School of Medicine obstetrical clinics and from JMH-Holtz Children's Hospital. Due to decreased number of eligible cases, some Zika-exposed infants were recruited retrospectively. In total, 38 mothers and infants were enrolled as study infants (Zika-infected or exposed) and 10 mothers and infants were enrolled as controls. Of the Zika-exposed, three cases had confirmed congenital Zika syndrome (CZS). Two were travel-associated, born to a Venezuelan and a Puerto Rican mother, respectively, who contracted Zika in their countries. One infant was born to a Miami native infected during the local outbreak; neither parent traveled outside of the country. The Venezuelan infant was the first reported case of CZS in the U.S. (2016). She was normocephalic at birth but had cerebral calcifications, retinal lesions, and abnormal tone. At 18 months, she had stable, non-vision-impairing retinal lesions, borderline low Bayley-lll language function, and unilateral hypertonicity. The other is the first case of non-travel-associated local transmission of CZS in the U.S. She has severe microcephaly, retinal lesions, severe visual impairment, hypertonicity, mild hearing deficit, and severe developmental delay at 12 months. The third infant, born in Puerto Rico, was brought to Miami after Hurricane Maria by his parents who initially consented to the study but returned to Puerto Rico thereafter. He has severe microcephaly, retinal lesions, hypertonicity and developmental delay.

   For the overall study sample, five infants have microcephaly, two with CZS and three with Zika exposure or indeterminant status. For the NNNS, preliminary data for the Zika-exposed group show higher rates of poor attention, atypically low arousal, and excitability on the NNNS compared to published full-term norms. On the Bayley-lll, eight of the 21 infants tested had at least one score 75 (two CZS, five Zika-exposed, one control). Thirty-two infants had at least one eye exam; 3 infants had lesions consistent with Zika and 1 infant had retinopathy of prematurity. The protocol included comparing Zika virus detection rates by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR; Florida Gulf Coast University Arbovirus Laboratory) in specimens of maternal and infant serum, urine, and saliva as well as maternal breast milk. However, none of the specimens tested positive by qRT-PCR, thus revealing that mothers need early, serial testing to capture viral shedding pattern and advance the epidemiology and science surrounding perinatal Zika infection.

   Preliminary results of this study, in collaboration with the other University of Miami Pediatric Zika Research Consortium research awards, support the need for early, definitive diagnosis of perinatal Zika exposure and long-term follow-up and early intervention for offspring with observed deficits.

   Follow On Funding: Center for Disease Control and Prevention/CMS - $1,025,000; Health Resources and Services Administration (HRSA) - $333,000

Collaborations: This research consortium evolved from the University of Miami's Zika Response Team and was working with the University of South Florida (Dr. Michael and Dr. Baker), Florida Gulf Coast University (Scott F. Michael, Ph.D. and Sharon Isern, Ph.D.), and the University of North Carolina (Dr. Julie A. Hofheimer).

Journals: Mittal, R., Nguyen, D., Debs, L.H., Patel, A.P., Liu, G., Jhaveri, V.M., Kay, S-IS., Mittal, J., Bandstra, E.S., Younis, R.T., Chapagain, P., Jayaweera, D.T., Liu, X.Z. (2017). Zika Virus: An Emerging Global Health Threat. Front. Cell. Infect. Microbiol. 7:486. doi:10.3389/fcimb.2017.00486.

Ventura, C.V., Bandstra, E.S., Fernandez, M.P., Cooper, J.M., Saigal, G.M., Bauer, C.R., Hofheimer, J.A., Berkovits, M.D., Fifer, R.C., Pensirikul, A.D., Gonzalez, I.A., Curry, C.L., Andreansky, S., Younis, R., Liu, X., Banker, T.P., Dubovy, S.R., Langer, S.M., Berrocal, A.M. (2018). First Locally Acquired Congenital Zika Syndrome Case in the United States: Neonatal Clinical Manifestations. Ophthalmic Surg Lasers Imaging Retina. 49: e93-e98. doi:10.3928/23258160-20180907-14.

Patents: None at the time of reporting.

9. **Grant #7ZK09:** Development of Nanoscale Approaches for Zika Virus and Therapeutics

   Principal Investigator: Nazira El-Hage, PhD

   Organization: Florida International University

   Progress Report: This consortium grant was formed by the Florida International University and the University of Miami investigators to focus on the entry of Zika virus (ZIKV) into the brain, neuropathology induced by the virus, and novel therapeutic approaches to protect against these events. The trafficking of ZIKV into the brain raises a question on the role of the blood-brain barrier (BBB) in this process. Dr El-Hage and associates hypothesize that ZIKV infects the brain via underdeveloped or disrupted BBB. This hypothesis was addressed by infecting pregnant mice at different stages of BBB development in embryos. Overall, their results showed that the Uganda strain of ZIKV-U (MR 766), the Asian lineages (a) Honduran strain ZIKV-H (R103451) and (b) Puerto Rican strain ZIKV-PR (PRVABC59) cause disturbances in the blood brain barrier by altering tight junction expression. These events may contribute to the dissemination of the viral infection in the central nervous system. The team also showed productive infectivity in human astrocytes and microglia with the different ZIKV strains and significant release of inflammatory responses that could lead to dysregulation in astrocytic function, metabolic disorders and brain abnormalities detected with ZIKV pathology. In terms of mechanism, they measured significant increase in the Toll Like Receptors (TLR)-3 and autophagy related proteins with all three strains of ZIKV while pharmacological and genetic inhibition of TLR3 caused a decrease in viral titers and in viral-induced inflammatory response in infected astrocytes. The researchers concluded that TLR3 plays a vital role in both ZIKV replication and viral-induced inflammatory responses, irrespective of the strains, while the autophagy protein Beclinl influences host inflammatory responses. In vivo studies using timed pregnant C57BL/6J mice and the autophagy deficient (B6.129X1-Been1tm1Blev/J) mouse model infected with the Uganda strain of ZIKV-U (MR 766), the Honduran strain ZIKV-H (R103451) or the (b) Puerto Rican strain ZIKV-PR (PRVABC59), resulted in growth retardation and underdeveloped brain in littermates born from autophagy deficient infected mice which correlated with a significant reduction in the expression levels of microcephaly related genes. On a separate note, receiving the Zika grant from Florida Department of Health has given the Herbert Wertheim College of Medicine the opportunity to be recognized as an outstanding research institution in the State of Florida. This

was evidenced by a subsequent report that was published in the Miami Herald on February 03, 2017. Data collected from this project was presented at the prestigious Keystone Symposium in Japan. Two research papers and one review paper related to Zika research are currently under review in peer reviewed journals.

Follow On Funding: None at the time of reporting.

Collaborations: Worked with the University of Miami (Dr. Michal Toborek and his team).

Journals: Pawitwar, S.S., Dhar, S., Tiwari, S., Ojha, C.R., Lapierre, J., Martins, K., et al. (2017). Overview on the current status of Zika virus pathogenesis and animal related research. J NeuroImmune Pharmacol. doi: 10.1007/s11481-017-9743-8.

Ojha, C.R., Rodriguez, R., Lapierre, J., Kumar, M.M., Branscome, H., Kashanchi, F., El-Hage, N. (2018). Complementary mechanisms potentially involved in the pathology of Zika Virus. Front. Immunol. doi: 10.3389/fimmu.2018.02340.

Patents: None at the time of reporting.

10. **Grant #7ZK10:** Development of a Diagnostic Assay for Rapid Detection and Quantification of Zika Virus.

    Principal Investigator: Waseem Asghar, PhD

    Organization: Florida Atlantic University

    Progress Report: Zika virus (ZIKV) has been found in blood, fueling growing concerns about the risk of transfusion-transmission with particular concern over severe outcomes in at-risk transfusion recipients such as pregnant women. Current ZIKV diagnosis assays are based on measuring early antibody immunoglobulin (Ig) M using enzyme-linked immunosorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR). There is a substantial serological cross-reactivity between ZIKV and other flaviviruses. Current IgM antibody-based ELISA assays cannot reliably distinguish between ZIKV and Dengue Virus (DENV). Therefore, an IgM positive result in a Dengue or Zika IgM ELISA test should be considered solely indicative of a recent flavivirus infection. Plaque-reduction neutralization tests (PRNT) can be performed to measure virus-specific neutralizing antibodies and can distinguish between infection by ZIKV and other flaviviruses. Although IgM ELISA followed by PRNT assay can identify the cause of viral infection, PRNT assays are time-consuming and take several days. RT-PCR based methods are complex, time consuming, and require multiple labor- intensive sample preparation and processing steps, hence not suitable for rapid testing at airports and point-of-care (POC) settings. To increase access to ZIKV testing and to reduce the disease spread, there is an urgent need to develop a reliable device for rapid ZIKV detection.

    The goal of this project was to develop a novel, low-cost (using transparency paper and plastic materials, <$2) and automated (on-chip virus lysis and impedance sensing) tool for rapid (~15 minutes) detection of ZIKV from human serum and saliva samples at POC settings. To develop a rapid Zika diagnostic assay, a panel of 16 different commercially available antibodies are tested for their specificity and reactivity to various strains of ZIKV envelop protein. SDS PAGE followed by western blot was performed to select specific antibodies that binds with ZIKV but does not react with DENV and Chikungunya virus. Selection of Zika specific antibodies is an important step to develop any diagnostic assay. To develop an impedance based sensor, electrodes were fabricated on paper based microfluidic device. These sensors were testing

and optimized for the buffer concentrations and experimental conditions used. They have also tested impedance of ZIKV samples and were able to identify ZIKV from DENV based on impedance sensing rapidly. Further, to automate the whole process, magnetic actuation platform was developed for automatic sample handling. Such a microfluidic chip design and actuation platform allow rapid detection of viruses at point-of-care settings.

Follow On Funding: None at the time of reporting.

Collaborations: This project involved two graduate students from Florida Atlantic University.

Journals: Herrada, C., Kabir, M., Altamirano, R., Asghar, W. (2018). Advances in Diagnostic Methods for Zika Virus Infection. ASME. J. Med. Devices. doi:10.1115/1.4041086.

Patents: None at the time of reporting.

11. **Grant #7ZK11:** Rapid RNA Test for Zika Virus

    Principal Investigator: Sapna K. Deo, PhD

    Organization: University of Miami

    Progress Report: Direct detection of Zika virus (ZIKV) RNA in patient samples is challenging and time consuming. The current strategy for ZIKV RNA detection is reverse transcription polymerase chain reaction (RT-PCR), and requires specialized laboratories and equipment. Additionally, no specific and sensitive immunoassays for ZIKV detection currently exist. This results in a significant delay in obtaining information on infection status, and may induce additional anxiety in pregnant women in Florida and around the world who remain at risk of infection. This necessitates the development of testing platforms for ZIKV that are simple, inexpensive, and rapid; can be easily mass-produced; and easily utilized in locations beyond traditional clinical settings. To solve this significant challenge, the researchers developed a portable, rapid detection technique employing rolling circle amplification of ZIKV RNA followed by paper-strip-based visual detection of the amplified product. Their preliminary data demonstrated that their technology works near room temperature and is capable of identifying the presence of ZIKV RNA on a paper strip using gold-nanoparticle conjugated probes that are observable with the naked eye. Moreover, because there is no need for specialized laboratory equipment, the entire test can be performed on-site without the need for any equipment or prior training. Successful detection of ZIKV infections in a rapid manner will meet the substantial need for assisting pregnant women in Florida and general population who currently have to wait several months to obtain results.

    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 #7ZK12:** Identification of Potent Neutralizing Zika Virus Antibodies Using Single-cell Analysis Technology

    Principal Investigator: Cuong Q. Nguyen, PhD

    Organization: University of Florida

    Progress Report: Transitioning from Africa to the Americas via the South Pacific, Zika virus

(ZIKV) infections became an emerging health pandemic of significant medical importance. Concern about ZIKV infections increased as the virus was linked to devastating neurodevelopmental defects in the newborns of infected pregnant women throughout the Americas. Over the past year, doctors in Brazil have documented over 4,000 cases of microcephaly in which infants were born with abnormally small heads. The detection of ZIKV in fetal brain tissues and anti-ZIKV antibodies in the mothers and/or infants established a possible causal link between ZIKV and microcephaly. Furthermore, there could be an additional link between ZIKV and the dramatic increase in the reported cases of Guillain- Barré syndrome. This rare disorder of the peripheral nervous system is characterized by muscle weakness and paralysis. The spread of ZIKV reached an alarming rate particularly in the state of Florida. Both the influx of travelers from ZIKV-infected areas and the warm tropical climate in this state promoted the survival of the ZIKV-carrying mosquitoes and accelerate the spread of the virus. Unlike other well-known flaviviruses like dengue, West Nile, and yellow fever viruses, there are no treatments or vaccinations against ZIKV, and diagnostic reagents are very limited. Although many investigations using immune-based therapies for arboviral infection have been pursued and have shown promise, there are no commercially available immune-based products for ZIKV. One critical challenge in the development of effective vaccines is their incomplete understanding of the protective humoral or antibody immunity against ZIKV. To meet this challenge, a team of experts used an innovative single-cell technology, referred to as single-cell antibody nanowells (SCAN), to quickly and efficiently screen individual B-cells for antigen specific products capable of neutralizing ZIKV. They adopted a plate-based neutralizing assay using flow cytometry. The neutralizing activity for each antibody was Zika strain-specific and dependent upon patient exposure to other flaviviruses. On-chip simultaneous detection of ZIKV and Zika-specific antibodies was accomplished using subnanolitre wells. Dr. Nguyen's team also transformed B cells of Zika-infected patients using Epstein-Barr virus. This lead to the successful cloning of B cell receptors of Zika-infected patients. With this data being submitted for publication, the investigators plan to resubmit a manuscript for on-chip detection of ZIKV and Zika antibodies. They also plan to screen for Zika activity and perform epitope mapping of neutralizing recombinant monoclonal antibodies (rmAbs) against ZIKV using shotgun mutagenesis and X-ray crystallography.

Follow On Funding: National Institute of Health - $417,897

Collaborations: None at the time of reporting.

Journals: Sahay, B., Nguyen, C.Q., Yamamoto, J.K. (2017). Conserved HIV Epitopes for an Effective HIV Vaccine. J Clin Cell Immunol. 4: 518. doi: 10.4172/2155-9899.1000518.

Voigt, A., Semenova, T., Yamamoto, J., Etienne, V., Nguyen, C.Q. (2018). Therapeutic Antibody Discovery in Infectious Diseases Using Single-Cell Analysis. In: Gu J., Wang X. (eds) Adv. Exp. Med. Biol. 1068: 89-102. doi: 10.1007/978-981-13-0502-3_8.

Patents: None at the time of reporting.

13. **Grant #7ZK13:** A Comparative Analysis of Zika Virus-induced Antiviral Response Mechanisms in Under-Studied Cell Populations

    Principal Investigator: Vladimir Beljanski, PhD

    Organization: Nova Southeastern University

    Progress Report: Cell type-based profiling of receptor tyrosine kinase (AXL) can readily explain

other ZIKV related conditions such as ocular defects, Guillain-Barre Syndrome, and sensory polyneuropathy, as well as predict effects on other biological systems with potentially long-term clinical manifestations. Blocking of AXL has been shown to almost completely prevent ZIKV infection. However, AXL signaling also plays critical roles in normal development and immunity, so targeting it directly would probably have multiple adverse consequences. Based on what is known about the effects of AXL signaling on immune responses, it is possible that in addition to using AXL as an entry receptor, ZIKV may also use it to enhance its own infectivity and suppress antiviral mechanisms. It is hypothesized that ZIKV infectivity can be reduced without interfering with cellular maturation processes by targeting the interface of AXL signaling and intracellular antiviral responses. This project tested the modulation of these pathways in a variety of AXL expressing cell types. Dr. Beljanksi's team was able to grow cells, test toxicity of candidate molecules, and started performing infection experiments during the summer months of 2017. Due to decreased experimental time, coupled with recent reports of cardiac impairments post ZIKV infections, the researchers were able to determine which antiviral pathways are involved in cellular defense against the virus. After these experiments, they decided to focus exclusively on fetal cardiac mesenchymal stromal cells (fcMSCs) as such cells have not been studied with respect to ZIKV infectivity and could be essential to understanding emerging reports that indicate cardiac impairment in subjects with ZIKV infection. Their data concluded that ZIKV infects and kills fcMSCs. Moreover, MSC markers did not change with ZIKV replication indicating that fcMSCs maintain their gene expression programs. The researchers plan to submit their findings for publication in the near future.

Follow On Funding: None at the time of reporting.

Collaborations: This project is in collaboration with Dr. Outi Hovatta from the Karolinska Institute, Sweden and a visiting professor at Nova Southeastern University.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

14. **Grant #7ZK14:** Longitudinal Brain MRI Characterization of Zika-Positive and Exposed Children Using Advanced MRI Techniques and Correlations with Neurodevelopmental Outcomes

    Principal Investigator: Gaurav Saigal, MD

    Organization: University of Miami

    Progress Report: The brain development of children who were prenatally exposed to Zika virus (ZIKV) or rather a Zika-exposed group [Zika polymerase chain reaction (PCR) and immunoglobulin M (IgM) negative and maternal PCR/IgM positive] is not yet known. Therefore, the goal of this project was to characterize longitudinal changes in the brain of children infected with or exposed to ZIKV using advanced brain imaging techniques, and correlate these changes to their neurodevelopmental outcomes. Of the 11 Zika-exposed infants, magnetic resonance findings were normal in 18 % (n=2) of patients and abnormal in the remaining 72% (n=9) of patients. The patients who demonstrated abnormalities showed a mild prominence of the cerebral spinal fluid spaces in the frontal and temporal lobes bilaterally; which was suggestive of possible mild volume loss of the brains of these children. Myelination pattern appeared normal in all the patients. There was questionable mild atrophy of the anterior corpus callosum in one of the infants. None of the infants demonstrated any abnormalities on the susceptibility weighted imaging sequence, to suggest any calcifications. Gyral pattern appeared normal in all patients as well. Some incidental findings (not specific to the ZIKV infection) were

noted in some of the infants.

One Zika-infected baby was scanned, and the findings include microcephaly as well as markedly simplified gyral pattern involving the supratentorial brain parenchyma. Due to the termination of Zika grant funding, screening, recruitment and enrollment was stopped a year and half after the start of the project.

Follow On Funding: Centers for Medicare & Medicaid Services (CMS) - $1,025,000

Collaborations: Jackson Memorial Hospital (three residents/fellows); a Neuroradiology fellow; a third-year postgraduate student; a fifth-year postgraduate student; and a pediatric Zika research consortium between various researchers at the University of Miami.

Journals: Ventura, C., Bandstra, E., Fernandez, M., Cooper, J., Saigal, G., Bauer, C., Hofheimer, J., Berkovits, M., Fifer, R., Pensirikul, A., Gonzalez, I., Curry, C., Andreansky, S., Younis, R., Liu, X., Banker, T., Dubovy, S., Langer, S., Berrocal, A. (2018). First Locally Acquired Congenital Zika Syndrome Case in the United States: Neonatal Clinical Manifestations. Ophthalmic Surg. Lasers Imaging Retina. 49: e93-e98. doi: 10.3928/23258160-20180907-14.

Mittal, R., Nguyen, D., Debs, L.H., Patel, A.P., Liu, G., Jhaveri, V.M., Kay, S-IS., Mittal, J., Bandstra, E.S., Younis, R.T., Chapagain, P., Jayaweera, D.T., Liu, X.Z. (2017). Zika Virus: An Emerging Global Health Threat. Front. Cell. Infect. Microbiol. 7:486. doi:10.3389/fcimb.2017.00486.

Mittal, R., Fifer, R.C., Liu, X.Z. (2018). A Possible Association Between Hearing Loss and Zika Virus Infections. JAMA Otolaryngol Head Neck Surg. 144(1):3–4. doi:10.1001/jamaoto.2017.1798.

Shiu, C., Starker, R., Kwal, J., et al. (2018). Zika Virus Testing and Outcomes during Pregnancy, Florida. USA, 2016. Emerging Infectious Diseases. 24(1):1-8. doi:10.3201/eid2401.170979.

Patents: None at the time of reporting.

15. **Grant #7ZK15:** Point of Sampling Rapid Detection of Zika and Other Mosquito-Borne Pathogens. Science, Technology, and Product Delivery

Principal Investigator: Barry W. Alto, PhD

Organization: University of Florida

Progress Report: The purpose of this project was to perform Zika virus and other arbovirus infection studies in cell culture and live mosquitoes to provide the necessary samples for testing the distributable device kit for detecting Zika virus in samples. To accomplish this goal, Dr. Alto's research team performed two aims: Aim 1: develop a universal sterilant based on ammonia-detergent mixtures and a pH indicator to assess the biohazard potentials of mosquito bodies prepared using their methodology, and Aim 2: Use a three-dimensional printer (Firebird) to convert an existing Breadboard Product into a distributable device. To address these aims, they used dilutions of virus prepared from cell culture and an infection experiment using Aedes aegypti mosquitoes and two emergent lineages of chikungunya virus (Asian and Indian Ocean lineages) were the basis to create arbovirus infected samples. Briefly, mosquitoes were provided with an infectious blood meal, underwent a 3 to 13-day incubation period, and then were tested for infection. Mosquito legs were dissected from bodies and tested by quantitative RT-PCR (CFX96 Real-Time PCR Detection System, Bio-Rad Laboratories) for chikungunya virus RNA to identify positively infected samples. The use of chikungunya virus is proof of concept that it works for all arboviruses, including Zika.

Making progress in addressing aim 2, three-dimensional printing of the arbovirus detection device was constructed into a distributable device kit for detecting Zika in trapped mosquitoes and other samples. The distributable device kit contained lyophilized reagent mixes with Reverse Transcription Loop Mediated Isothermal Amplification (RT-LAMP) primers, deoxynucleotide (dNTP) mixture with dUTP, strand displacing DNA polymerase, a reverse transcriptase, RNase inhibitor (to protect viral RNA from degradation), a thermolabile uracil DNA glycosylase (to prevent forward contamination by digesting dU-containing amplicons from previous assays), a lyoprotectant (to benefit long-term storage of the reagents), and was supplemented with a rehydration buffer, Q-paper, positive controls, ammonia solution, and an Observing Box. The detection kit has been distributed to Florida Mosquito Control Districts (FMCD) along with in-house demonstration and instruction at each of the FMCD visited. A detection kit and several reagents (that allow for a low multiplex test including Zika, dengue, and chikungunya viruses) were left with each FMCD for further testing and feedback.

Follow On Funding: None at the time of reporting.

Collaborations: This project has worked with the Indian River State College (an undergraduate student), Florida Medical Entomology Laboratory and several Florida Mosquito Control Districts.

Journals: Glushakova, L.G., Alto, B.W., Kim, M.S., Burkett Cadena N.D., Wiggins, K., Eastmond, B., Benner, S.A. (2018). Optimization of cationic (Q)- paper for detection of arboviruses in infected mosquitoes. J. Virol. Methods. 261:71-79. doi: 10.1016/j.jviromet.2018.08.004.

Yaren, O., Alto, B. W., Bradley, K. M., Moussatche, P., Glushakova, L., Benner, S. A. (2018). Multiplexed Isothermal Amplification Based Diagnostic Platform to Detect Zika, Chikungunya, and Dengue 1. J. Vis. Exp. 133: e57051, doi:10.3791/57051.

Patents: None at the time of reporting.

16. **Grant #7ZK16:** Fetal Brain Exosomes in the Maternal Circulation for the Detection of Zika Virus Infected Fetuses

Principal Investigator: David G. Meckes Jr., PhD

Organization: Florida State University

Progress Report: Zika virus was an emerging infectious disease that had spread rapidly across the Caribbean and South America with over 240 confirmed cases of locally-acquired Zika in the United States. Zika is an enveloped, positive-sense, single-stranded RNA virus that is a member of the Flaviviridae family. Infection of pregnant women during the first trimester has been linked to microcephaly, a neurological condition where babies are born with significantly smaller heads due to abnormal brain development. Babies born with microcephaly can develop convulsions and suffer physical and learning disabilities that persist into adulthood. Accumulating evidence supports an important role of extracellular vesicles in the progression of neurological conditions and the spread and pathogenesis of infectious diseases. Extracellular vesicles (EVs) are membrane-encapsulated structures released by cells of endosomal (exosomes) or plasma membrane (microvesicles) origins. EVs carry signaling factors, proteins and microRNAs that mediate intercellular communication. It has been demonstrated that EVs from Hepatitis C virus (HCV) infected individuals and cells contain replicative-competent viral RNA that was capable of infecting hepatocytes. Being a member of the same viral family, it is likely the Zika virus also hijacks EV pathways to package viral components and secrete vesicles that are infectious and potentially less immunogenic. As EVs have been shown to cross blood-brain and placental

barriers, it is also possible that Zika virus could usurp normal EV biology to gain access to the brain or developing fetus. During the funding period, Zika virus infected cells were discovered to secrete EVs that are distinct from virus particles with specific viral protein profiles and infectious genomes. Interestingly, infection resulted in a massive increase in EV numbers which exhibited altered densities and an enrichment of CD9 when compared to EVs from uninfected cells. These Zika virus-modified EVs were capable of infecting cell types important to pathogenesis and spread in humans. Analysis of inflammatory cytokine production induced in macrophages exposed to Zika EVs or virions revealed that EVs induce lower levels of pro-inflammatory cytokines when compared to virions. Overall, these findings provide evidence for a novel means of Zika virus transmission that may have implications in understanding how the virus crosses cellular barriers to infect the developing fetal brain. Furthermore, the molecular content in EVs released from infected cells determined from prior studies may serve as novel biomarkers for the detection of Zika virus infection.

During the grant period, a new method was developed to separate exosomes from virions combining polymer-based precipitation with recently described Optiprep density flotation gradients. With this approach, Dr. Meckes' team determined for the first time that Zika virus dramatically alters exosome numbers, density, and content compared to control, "healthy" exosomes. These findings support the hypothesis that the unique properties of exosomes present in the circulation can be utilized for diagnostic purposes. It was also determined that Zika virus-modified exosomes contain viral RNA and glycoprotein in the absence of capsid similar to what has been described for other Flaviviruses. Therefore, these viral modified exosomes may support an alternative means of mediating infection or enhancing virus pathogenesis in neighboring cells. The lab is currently pursuing these lines of investigation.

Methods were also optimized for purifying extracellular vesicles in plasma samples using size exclusion chromatography (SEC) and compared results obtained to other commonly used vesicle isolation methods used in the field. SEC was found to be superior to other methods with regards to yield and purity. The researchers have gone on to purify plasma-derived exosomes using this method and started analyzing exosmal RNAs with RNA-Seq. These methods will be applied to compare the miRNAs and mRNAs in exosomes isolated from Zika-infected and non-infected cells. This data may help provide targets to validate in human blood samples.

Follow On Funding: None at the time of reporting.

Collaborations: Universidad Central Del Caribe and University of Puerto Rico.

Journals: Sun, L., Meckes, D.G. Jr. (2018). Methodological Approaches to Study Extracellular Vesicle miRNAs in Epstein–Barr Virus-Associated Cancers. Int. J. Mol. Sci. 19(9):2810. doi:10.3390/ ijms19092810

Patents: None at the time of reporting.

17. **Grant #7ZK17:** Zika Virus Activation and Inhibition of Human Complement Immunity

Principal Investigator: Griffith D. Parks, PhD

Organization: University of Central Florida

Progress Report: The deleterious health effects of Zika can be clearly seen because it evades the human immune system, and subsequently affects a large number of other human organ systems including reproductive, cardiovascular, developmental, and central nervous systems. All

viruses (including Zika virus) must face normal pre-existing immune responses in the human host known as “innate immunity.” One of the strongest of these innate immune systems in humans is called Complement, which is a series of human proteins that recognize viruses and infected cells to inactivate them. The Complement system is a critical front-line defense against viruses, but also acts to modulate the formation of later protective immunity such as antibodies and immune cells. In the ongoing laboratory studies with Zika virus, the Dr. Park’s and his team hypothesize that Zika virus has novel mechanisms to resist neutralization by human serum, but the effectiveness of this inhibition varies from person to person. In addition, they also suggested that there may be human factors that differ between individuals that can control whether they can inactivate Zika virus or whether it spread in the body. The team tested serum components to elucidate the interactions of Zika virus (ZIKV) with human innate immune systems and how the virus inhibits these pathways to survive and spread through the host. They developed protocols to purify three ZIKV strains (MR766, IBH and PRVABC59) from insect cells and from human lung epithelial cells. A key finding that drove the overall hypothesis for this project was the preliminary data showing that sera from individual donors differed in their capacity for complement-mediate neutralization of Zika in vitro. The findings of the complement component C6 subunit and C1q suggested that the neutralization by donor #3 was proceeding because the flavivirus nonstructural protein 1 (NS1) cannot bind to and inactivate C1q. This was supported by experiments where soluble NS1 was not associated with C1q from the donor #3 subject. These findings supported the hypothesis that for the donor #3 serum which can neutralize, there was a C4-dependent activation of complement pathways which ultimately resulted in the formation of the membrane attack complex. This was driven by C1q-dependent activation. In subsequent grant applications, the research team plans to titrate the mosquito salivary proteins (MSP) extract to determine if they can find binding of a subset of proteins. This next step is crucial before processing the data using proteomics analysis.

Follow On Funding: None at the time of reporting.

Collaborations: This project involved investigators from Nemours Children’s Hospital at Lake Nona and the University of Central Florida College of Medicine.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

18. **Grant #7ZK18:** Rapid Diagnostic Test for Zika Virus in Dried Blood Spots with Low Demands on Instrumentation

    Principal Investigator: John G. Morris Jr., MD

    Organization: University of Florida

    Progress Report: To address the challenges of diagnosing Zika in a rapid and cost-effective manner, Dr. Morris and his team proposed to diagnose Zika virus (ZIKV)-infection using specimens in the form of dried blood spots coupled with reverse transcription strand invasion based amplification (RT-SIBA). Unlike the common practice of spotting blood onto Flinders Technology Associates (FTA) filter paper (“FTA cards”), researchers at the Centers for Disease Control and Prevention(CDC) report that for blood-borne ribonucleic acids (RNA) viruses, superior ZIKV genomic RNA (vRNA) detection occurs using blood spotted onto high-quality filter paper instead of FTA cards. The researchers most significant findings were: (a) Acute-phase blood for ZIKV reverse transcription polymerase chain reaction (RT-PCR) tests, spotted onto high quality chromatography paper filters to form dried blood spots (DBS), can be stored at room

temperature for at least one month without compromising their usefulness as test specimens, and (b) reverse transcription isothermal amplification (RT-LAMP) is a cost-effective, fast, and sensitive diagnostic tool for the detection of vRNA.

Whereas high-quality chromatography paper is a superior substrate for the preparation of DBS, there is a disadvantage: blood-borne pathogens are not necessarily inactivated when blood is spotted onto the filters. This poses biohazard concerns, especially if the filters containing DBS must be mailed to remote testing sites. The work conducted revealed that DBS prepared in Flinders Technology Associates (FTA) cards were a suitable alternative. In particular, FTA cards contain chemicals that inactivate many viruses and at the same time, stabilize their genomes, making vRNA extracted from the DBS suitable for future quantitative real-time RT-PCR or RT-LAMP detections of ZIKV vRNA. For the purpose of ZIKV vRNA detection, DBS packaged with desiccant can be stored for at least 1 month at room temperature without significant loss of RT-PCR or RT-LAMP signal sensitivity. When sufficient ZIKV vRNA is present, as would be expected during the acute phase of infection, ZIKV vRNA can be detected by RT-LAMP in as little as 8 minutes and up to 25 minutes. Apart from DBS, RT-LAMP is a promising low-cost method for detecting ZIKV vRNA extracted from a minimal volume of whole blood at the site of care, with minimal needs for instrumentation.

The researchers tested clinical specimens collected in Venezuela during the 2016 ZIKV outbreak to explore whether their findings were relevant for real-world specimens. Of the 72 specimens collected, 36 were positive for ZIKV after two years of storage on FTA cards, further suggesting that ZIKV remains stable in DBS on FTA cards.

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.

19. **Grant #7ZK19:** University of South Florida Integrated Clinical Trial Network Structuring and Enhancement for Execution of Zika Virus Vaccine and Diagnostic Clinical Trials, and Testing of Other Emerging Infectious Disease (EID) Solutions for Florida

    Principal Investigator: Thomas B. Casale, MD

    Organization: University of South Florida

    Progress Report: As mosquitoes that transmit Zika virus (ZIKV) are widespread throughout much of the USA and especially Florida, the ZIKV presented a severe and possibly persistent health risk to the residents of Florida (especially pregnant women). Thus, building clinical trial network capacity for ZIKV vaccines and diagnostics is critical to stem the tide of this rising health crisis both in Florida and around the world. Such a network is also urgently needed to address similar issues with other emerging infectious diseases (EID) in the region surrounding Florida. The overall goal of this project was to develop a clinical trials network. Specific Aims: 1) To amalgamate and strengthen key relevant University of South Florida (USF) clinical trials teams into a Zika Clinical Research Network (ZiCRN) in Florida, with the purpose of testing drugs, vaccines and diagnostics in response to the current ZIKV emergency and other EID. This program will leverage expertise from academic, government and industry experts to achieve a multifunctional, integrated approach to rapidly and effectively execute human clinical trials in

response to these needs; and 2) To enhance training about clinical research, global health, and biotechnology expertise within the USF system to better address the ZIKV emergency and future emerging infectious diseases crises. Dr. Casale's research team made considerable progress in these aims. Five major groups at the University of South Florida College of Medicine began a strong collaboration as a result of this funding award; The Division of Allergy and Immunology in the Department of Internal Medicine, The Division of Infectious Diseases in the Department of Internal Medicine, the Departments of Global Health and Environmental and Occupational Health and the Florida Department of Health. This collaboration led to a united and concerted efforts to pursue clinical and diagnostic trails for emerging infectious diseases with an emphasis on Zika. In addition, they have fostered basic research programs, diagnostic laboratory experience and clinical research experience aimed at training the next generation of investigators working on emerging infectious diseases and immunology. This included both classroom and practical training across the college of medicine and college of public health at the University of South Florida.

Follow On Funding: None at the time of reporting.

Collaborations: This research involved the USF Medicine International with USF Health Panama partners, USF Health Ecuador partners, Morsani College of Medicine, the Department of Global Health and Center for Global Health and Infectious Diseases Research (GHIDR), and the Gorgas Memorial Research Institute of Health Studies (a USF Health International partner). With the help of the Florida Department of Health sponsored Zika research symposium, the team also collaborated with Dr. Gerasimova from the University of Central Florida. The University of Cartagena in Colombia was also noted as a possible collaborating entity.

Journals: Morano, J.P., Holt, D.A. (2017). The social determinants of health contextualized for the Zika virus International Journal of Infectious Disease. 65: 142-143. doi: 10.1016/j.ijid.2017.10.006.

Casale, T.B., Teng, M.N., Morano, J.P., Unnasch, T., Lockwood, C.J. (2018). Zika Virus: An Emerging Infectious Disease with Serious Perinatal and Neurologic Complications. J. Allergy Clin. Immunol. 141(2): 482-491. doi: 10.1016/j.jaci.2017.11.029.

Patents: None at the time of reporting.

20. Grant #7ZK20: Early Diagnosis and Rehabilitation for Craniofacial Disorders in Congenital Zika Syndrome

Principal Investigator: Ramzi T. Younis, MD

Organization: University of Miami

Progress Report: Throughout the life of this grant, 48 diagnostic evaluations were conducted on 26 enrolled infants and toddlers. Diagnostic testing consisted of a combination of auditory evoked potentials, distortion product otoacoustic emissions, tympanometry, and behavioral visual reinforcement audiometry. The test selection between auditory evoked potentials and behavioral visual reinforcement audiometry was determined by the child's age, level of developmental maturation, and behavioral responses to environmental sounds. Both techniques served the same purpose of determining minimum levels of hearing sensitivity looking for evidence of possible hearing loss that could be associated with Zika virus (ZIKV) infections. In addition to providing information regarding hearing sensitivity, the auditory evoked potentials procedure examined the status of the auditory central nervous system along the auditory nerve and upward through the brainstem. Consequently, the researchers examined hearing sensitivity (auditory

evoked potentials or behavioral visual reinforcement audiometry), middle ear status (tympanometry), the health of the sensory cell system of the cochlea (distortion product otoacoustic emissions), and the integrity of the auditory pathway within the central nervous system (auditory evoked potentials). Diagnostic evaluation outcomes were normal for all but two children. Using this data, the reach team calculated a sample estimate of incidence by performing a record review of 14 children not enrolled in the study simply to offer a larger sample by which to perform the incidence estimate calculation. Out of a sample pool of 40 children, the investigators found evidence of hearing loss in two infants offering an incidence estimate of 2.5% in their sample. This compared to 1.5 per thousand found to have hearing loss among the general population of infants in the State of Florida (Source: Children's Medical Service report to Genetics and Newborn Screening Advisory Council, August 25, 2017).

The observations of the hearing losses in these two cases were, in their opinion, more significant than simply the incidence of occurrence. One child (case Z005) had significantly low frequency hearing loss rising to normal hearing sensitivity in the mid- and high-frequencies. Having low frequency hearing loss such as this, can result when associated with a brainstem site of disorder. This observation may influence the protocols that determine the presence of auditory problems. The CDC recommends use of auditory evoked potentials with a "click" stimulus as a screening tool for detection of hearing related issues (Adebanjo et al., 2017). Further findings from case Z005 indicates that only a full diagnostic protocol incorporating low-frequency tone bursts will be essential in order to recognize children with auditory issues whether they be of cochlear origin or of central nervous system origin.

The second child (case Z001) displayed significant unilateral hearing loss from test to retest for approximately one year. At the most recent evaluation, hearing sensitivity had reverted to normal threshold levels. This observation is very significant in that it offers insight to the mechanism of action of ZIKV. First, it demonstrates that the cochlea could not be the site of disorder for the hearing loss. Once human sensory cells are destroyed or significantly compromised to produce hearing loss, they never recover. Consequently, the site of origin most certainly was the lower brainstem in the region of the pons.

Despite the small sample size of the study, the proportion of children with hearing loss demonstrated an almost 17-fold increase from what is seen within the general population of newborns in Florida. The conclusion from these observation and corroborating studies indicate that the risk of auditory disorders may extend well into childhood because of the implications of both peripheral and central sites of involvement. As such, these children need to be observed on a regular basis over time with both standard audiometry and auditory evoked potentials to monitor for the possibility of ZIKV related auditory problems.

Follow On Funding: Health Resources and Services Administration (HRSA) - $333,000

Collaborations: Doctor of Audiology students from four universities participated in this research project: Rush University, Pacific University, Syracuse University, and the University of Puerto Rico. Other institutions that were involved were the University of Miami (Dr. Gonzalez, Dr. Bandstra, Dr. Bauer, Dr. Gaurav, Dr. Curry, Dr. Berrocal, and Dr. Lim), Virgin Islands Department of Health, the University of the Virgin Islands, Universidade Federal de Pernambuco and Hospital Agamenon Magalhaes (Dr. Leal).

Journals: Mittal, R., Fifer, R.C., Liu, X.Z. A Possible Association Between Hearing Loss and Zika Virus Infections. *JAMA Otolaryngol. Head Neck Surg.* 2018;144(1):3–4. doi:10.1001/jamaoto.2017.1798

Ventura, C., Bandstra, E.S., Fernandez, M., Cooper, J., Saigal, G., Bauer, C., Hofheimer, J., Berkovits, M., Fifer, R., Pensirikul, A., Gonzalez, I., Curry, C., Andreansky, S., Younis, R., Liu, X., Banker, T., Dubovy, S., Langer, S., Berrocal, A. First Locally Acquired Congenital Zika Syndrome Case in the United States: Neonatal Clinical Manifestations. Ophthalmic Surg Lasers Imaging Retina. 2018; 49: e93-e98. doi: 10.3928/23258160-20180907-14.

Mittal, R., Nguyen, D., Debs, L.H., Patel, A.P., Liu, G., Jhaveri, V.M., Kay, S., Mittal, J., Bandstra, E.S., Younis, R.T., Chapagain, P., Jayaweera, D.T., Liu, X.Z. (2017). Zika Virus: An Emerging Global Health Threat. Front. Cell. Infect. Microbiol. 7. e486. doi:10.3389/fcimb.2017.00486.

Patents: None at the time of reporting.

21. Grant #7ZK21: Evaluation of Novel Vaccines that Prevent Zika Infection

Principal Investigator: Glen N. Barber, PhD

Organization: University of Miami

Progress Report: In 2015, outbreaks of Zika virus (ZIKV) were reported for the first time in Brazil and were associated with abundant causes of microcephaly as perceived in aborted fetuses and in infants born to ZIKV infected mothers. For example, Brazil normally reports approximately 150 cases of microcephaly per year. However, in 2015 alone, approximately 3000 cases were documented, which manifests a raise from 5.7 to 99.7 cases per 100,000 births. ZIKV was detected in South Florida with numerous documented cases of infection occurring in the Miami region. Besides being transmittable by mosquito, however, ZIKV has now been documented as being sexually transmittable. There are presently no therapies or vaccines to treat or prevent ZIKV infection, respectively, and thus the development of such measures is naturally of paramount importance. Consequently, Dr. Barber and associates developed novel, effective vaccines that may protect against ZIKV infection. The goal was to further test the effectiveness of their vaccines with the objective of generating sufficient data to warrant the consideration of clinical trials. Recombinant Vesicular Stomatitis virus (rVSV) has been shown to be a highly effective and safe vector for the delivery of foreign immunogens for vaccine purposes. They have generated rVSVs (two types VSV and VSVΔM) that express either the full length ZIKV envelope antigen (env) protein incorporating the transmembrane (TM) region (VSV-ZprME) or a truncated version of the ZIKV env that lacks the TM (VSV-ZENV) and have evaluated their immunogenicity in murine models. Both the ZIKV prME and ENV were efficiently expressed by rVSV's; they detected antibody produced to both ZENV proteins were readily generated following the inoculation of mice, including neutralizing antibody and cytotoxic T cells.

Significantly, the offspring of their vaccinated female mice exhibited resistance to ZIKV infection following challenge; therefore, the recombinant Zika vaccines elicited the desired results. The research team was able to produce these vaccines at the desired concentration and purity to start the nonhuman primate experiments using the pregnant pigtail macaque model of ZIKV infection. They have enrolled a cohort of pigtail macaques into their vaccination study. The animals were screened via serology for previous exposure to ZIKV, West Nile virus, yellow fever virus, and dengue virus, as well as to specific parasites. The macaque cohort was deemed negative to all microbes tested and has had no prior exposure to flaviviruses. The researchers have now started the inoculation of rVSV-ZIKV vaccine into the macaques. Preliminary results indicate that VSVΔM-ZprME is also able to elicit an immune response against ZIKV, as a consistent increase in immunoglobulin M titer against ZENV is observed following prime

vaccination. They plan to use their data for the design of future studies, potentially using nonhuman primates or even for clinical trials.

Follow On Funding: None at the time of reporting.

Collaborations: Department of Immunology in partnership with the Department of Obstetrics and Gynecology at the University of Washington Seattle.

Journals: Betancourt, D., de Queiroz, N.M., Xia, T., Ahn, J., Barber, G.N. (2017). Cutting Edge: Innate Immune Augmenting Vesicular Stomatitis Virus (VSV) expressing ZIKA Virus Proteins Confers Protective Immunity. J Immunol. Apr 15; 198 (8) 3023-3028. doi:10.4049/jimmunol.1602180.

Patents: VSV-ZIKA Virus Vector for Treating Zika Virus Infection. Patent No. PCT/US2017/031067

22. Grant #7ZK22: Multiplexed Detection Platform for Point-of-Service Testing of Zika Virus

Principal Investigator: Hugh Z. Fan, PhD

Organization: University of Florida

Progress Report: Zika virus (ZIKV) is a mosquito-borne RNA virus and it is a major public health concern primarily because it has been linked to microcephaly in newborns. Since the current methods authorized for assessing ZIKV infection are carried out in laboratories, the researchers have successfully developed laminated paper-based analytical devices (LPAD) for ZIKV detection at the point of care or in the field.

The most significant scientific accomplishments throughout this project is the development of a sample preparation unit and LPAD detection unit. For the sample preparation unit, Dr. Fan's research team developed a novel valve concept that could allow virus lysis, RNA enrichment, and purification in an automatic fashion. For the detection unit, research staff employed an isothermal nucleic acid amplification for genetic identification of ZIKV, followed by colorimetrical reading by naked eye. These two units have been integrated into one platform that is portable, easy to operate, and of low cost. The researchers demonstrated the platform using as low as 0.1 PFU (plaque-forming units) of ZIKV in water and 0.5 PFU in saliva or urine.

The sudden termination of the Zika Research Grant Initiative in the middle of the project significantly affected the realization of the overall objective of the original project. With resources shifting to this project during the last couple of months of the program, research staff were able to make significant progresses that enabled them to file a patent application.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Cassano, C.L., Georgiev, T., Fan, H.Z. (2017). Using Airbrushes to Pattern Reagents for Microarrays and Paper-fluidic Devices. Microsystems & Nanoengineering. 3.17055. doi:10.1038/micronano.2017.55.

Garcia-Cordero, J.L., Fan, H.Z. (2017). Sessile droplets for chemical and biological assays. Lab on a Chip. 17. 2150-2166. doi:10.1039/C7LC00366H.

Patents: Provisional patent application entitled "Apparatus and Method for Performing

Microorganism Detection" filed on July 31, 2018. Application No. 62712571.

23. **Grant #7ZK23:** Cellular and Molecular Mediators of Zika Virus Replication in Decidua and Mechanisms of Zika Virus Transmission from Maternal Decidua to the Placental/Fetal Unit

Principal Investigator: Charles J. Lockwood, MD

Organization: University of South Florida

Progress Report: Flaviviruses are enveloped, positive-stranded ribonucleic acid (RNA) viruses that are an emerging global health threat. They include dengue, yellow fever, Japanese encephalitis, St. Louis encephalitis, tick-borne encephalitis, West Nile and Zika Viruses. In pregnant women, the impact of mosquito-transmitted Zika virus (ZIKV) infection on the mother is usually minimal except in cases post-viral Guillain–Barré syndrome. However, in the fetus the virus can cause severe developmental problems, ranging from fetal growth restriction, chronic placentitis and/or severe congenital defects. Preliminary observation suggests that the virus can evade local immune barriers in the placenta and brain to infect these tissues which in turn act as reservoirs causing long term shedding of the virus. However, it is unclear how the virus gains access to the placenta and subsequently to the fetus. Such information is vital to prevent these catastrophic outcomes. The placenta is attached to the uterine decidua. The latter is a specialized tissue composed of equal number decidual cells (50%) and decidua-specific immune cell types including uterine natural killer cells (60-80%), macrophages (20-25%) and T-lymphocytes/ dendritic cells (1-2%). The decidua is the only site of direct cell-cell interactions between the maternal and fetal tissues (aka the maternal-fetal interface). Dr. Lockwood's research team posits that decidua-mediated immunosuppression enables ZIKV survival, replication and dissemination into the placenta. Thus, containment and eradication of ZIKV in the decidua should prove crucial to the prevention of subsequent placental transmission. Tizoxanide, an anti-viral agent, was evaluated for its role in preventing ZIKV infection in cultured first and term decidual cells (FTDCs and TDCs) and human endometrial stromal cells (HESCs) infected with a ZIKV strain. ZIKV-infected Møs and dendritic cells (multiplicity of infection=1), were treated with tizoxanide. The results from this project indicated that HESCs, FTDCs, and TDCs are highly permissive to ZIKV infection in a trimester-dependent manner, and that all three maternal cell types likely serve as reservoirs for placental/fetal infection. This observation also helps explain why pregnant women are particularly susceptible to ZIKV infection in the first trimester. Taken together, these findings support the researchers' hypothesis that permissiveness of ZIKV infection in these maternal cells correlate with expression levels of viral entry molecules. Thus, the efficacy of tizoxanide in inhibiting ZIKV replication in infected maternal cells suggest its use in preventing/reducing perinatal transmission of this detrimental virus. Due to the withdraw of Zika funding, ongoing studies will be examining ZIKV replication in cell lysates and supernatant by measuring ZIKV RNA using qualitative-PCR. Parallel studies of the effects of tizoxanide on chemokine production in ZIKV-infected Møs and dendritic cells are in progress.

Follow on Funding: None at the time of reporting.

Collaborations: This project would not have been possible without Dr. Ozlem Guzeloglu-Kayisli, the Department of Obstetrics & Gynecology and Rep. Science from Yale University (Dr. Seth Guller), and the Romark Laboratory.

Journals: Casale, T.B., Teng, M.N., Morano, J.P., Unnasch, T., Lockwood, C.J. (2018). Zika Virus: An Emerging Infectious Disease with Serious Perinatal and Neurologic Complications. J. Allergy Clin. Immunol. 141(2): 482-491. doi: 10.1016/j.jaci.2017.11.029

Patents: None at the time of reporting.

24. Grant #7ZK24: Cardiovascular Complications Related to Zika Virus Infection

Principal Investigator: Claudia Martinez, MD

Organization: University of Miami

Progress Report: Evidence from outbreaks of other flaviviruses within the same family of the Zika virus have reported cardiovascular complications in a significant number of infected patients. Because Zika virus is one of the flaviviruses that manifest with systemic infection, there is a risk for cardiovascular involvement either by direct organ damage or indirectly through a secondary inflammatory response. Therefore, infection-related anomalies may be present in both cardiac structure and function as well as in the vasculature. The University of Miami has established a Zika Global Network assembled with both clinical and basic researchers, which includes an Infectious Disease team of faculty and staff who are dedicated to evaluating and managing the health care of all potential Zika-infected patients and those patients confirmed seropositive.

The most significant scientific accomplishment of the "Zika Heart" project was the implementation of the research project soon after it was awarded, and the enrollment of 4 subjects within the first two quarters of 2017. Three of the subjects had documented prior Zika infection. Of these three subjects, one had been concomitantly infected with Dengue, and was therefore excluded from further assessment, per protocol eligibility requirements. Another was excluded due to the timing of her Zika testing. One Zika positive patient, and one Zika negative patient completed both episodes of the study- the baseline testing done in 2017, and the final assessments which were completed 12 months later in 2018. One of the subjects (zika+) demonstrated signs suspicious of myocarditis, as per cardiac magnetic resonance imaging results and another subject (zika -) demonstrated diminished fibromuscular dysplasia at 12 months follow up.

As a result of the strong collaborative environment within the Zika Network team at the University of Miami, four other subjects were identified as potential candidates due to their Zika serostatus, but they were not enrolled due to concomitant documented dengue exposure, or exceeding the age criterion. The research collaboration that emerged from this project forged strong research relationships between clinicians and researchers from different departments, including Cardiology, Behavioral Science, Infectious Disease, Radiology, Center for Translational Research at the University of Miami, as well as with Florida International University.

Follow on Funding: None at the time of reporting.

Collaborations: The University of Miami (between Departments of Cardiology, Infectious Disease, Behavioral Science, Radiology, and the Center for Translational Research) as well as the Florida International University.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

25. Grant #7ZK25: Identifying Molecular Targets for Spatial Mosquito Repellent Design

Principal Investigator: Matthew J. DeGennaro, PhD

Organization: Florida International University

Progress Report: Current approaches to control the spread of Zika are ineffective. Aedes

aegypti, the principal vector of Zika, is very difficult to eradicate because it is so well adapted and has shown resistance to insecticides. Using insect repellents is one of the few tools to protect against mosquito bites and the subsequent transmission of diseases. The current EPA approved repellents: picaridin, IR3535, oil of lemon eucalyptus (OLE), and DEET (diethyltoluamide) are most effective when worn on skin, but are not fully protective. None of these chemicals are particularly good spatial repellents, so cannot repel mosquitoes at distances sufficient to prevent mosquitoes from entering homes or outdoors spaces. The project goal was to identify the genes that allow mosquitoes to smell repellents to facilitate the design of new mosquito repellents. Dr. DeGennaro's research team has developed a technique that allows them to figure out which genes "smell" an odor by assessing how the olfactory receptor gene expression is altered after a mosquito is exposed to that odor. They exposed mosquitoes to the insect repellents picaridin, IR3535, oil of lemon eucalyptus (OLE), para-menthane-diol (PMD), and DEET. Based on their research findings, they have shown that their method of looking at mRNA levels in mosquitoes can reveal reductions in the expression of cognate olfactory receptors when exposed to their odor-ligands. This is the first time this has been shown in a mosquito. The researchers have been able to expand their assay to allow for testing a large number of mosquitoes at once. This advance allows them to do RNA-sequencing to look in an unbiased way at the effects of repellents on the mosquito olfactory system. They have completed the behavioral and molecular experiments to discover which repellents activate which olfactory receptors using RNA-sequencing. Currently, they are waiting for the next generation sequencing data to be returned for analysis.

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.

26. Grant #7ZK26: ZIK-Action: Evaluation of Infants for Zika Related End Organ Damage, a Time Science Approach

Principal Investigator: Ivan A. Gonzalez, MD

Organization: University of Miami

Progress Report: Infants exposed to Zika in utero have the potential to develop Congenital Zika Syndrome (CZS); yet no data have been published on this subset of infants regarding their development. Thus, the goal of this project was to analyze Zika-associated pathologies in infants and correlate such outcomes with infant and maternal biomarkers (inflammatory and immunologic) in order to establish predictors of CZS. Preliminary anthropometric data has been collected for each visit.

The extent of end organ damage in infected and exposed newborns were measured at each visit by ocular, cardiovascular and renal ultrasounds, and neurological deficits by encephalogram. Additionally, Dr. Gonzalez's team performed immune responses to the virus by serology to define the immunological changes in maternal cohort during the time of recruitment. Investigators were blinded to serological status of maternal Zika virus exposure during the pregnancy and the outcome in their newborn infants. Research findings have suggested that patients in this cohort were found to have an increased incidence of echo-cardiac findings. Analyses are in progress with cardiovascular markers as surrogates to cardiac findings. Preliminary analysis does not

show correlation. Also, congenital Zika virus (ZIKV) infection appears to reduce by total kidney volume. This data being evaluated in the context of renal function by analyzing the levels of Cystatin C in serum, creatinine/albumin ratio in plasma, and urine analysis. Preliminary analysis is inconclusive. Ocular ultrasounds were normal in confirmed and suspected CZS cases. In spite of positive retinal findings, the structure reveals no abnormalities. A comprehensive ophthalmic examination revealed normal anterior segment structures and pupil reaction to light with no afferent pupillary defect bilaterally. Fundoscopy showed bilateral increased cup-to-disk ratio, pigment mottling and sharply demarcated chorio-retinal atrophy within the macular regions. Therefore, no ultrasound findings were discovered. Other findings suggested that maternal serum antibody (Ab) responses of the cohort mothers demonstrated approximately 90% Ab reactivity to ZIKV by enzyme-linked immunosorbent assay (ELISA), whereas approximately half of the cohort had neutralizing Abs against ZIKV by plaque reduction neutralization test (PRNT) assay. The researchers also successfully established which cohort participants had prior infections with ZIKV versus dengue virus (DENV). The preliminary analysis demonstrated a 50% distribution between ZIKV and DENV.

Follow on Funding: Centers for Disease Control and Prevention/ Centers for Medicare & Medicaid Services - $1,025,000

Collaborations: This project worked with the Florida Chapter of the American Academy of Pediatrics and is using funding from a CDC/CMS grant to establish a referral center for South Florida community.

Journals: Ventura, C.V., Bandstra, E.S., Fernandez, M.P., Cooper, J.M., Saigal, G.M., Bauer, C.R., Hofheimer, J.A., Berkovits, M.D., Fifer, R.C., Pensirikul, A.D., Gonzalez, I.A., Curry, C.L., Andreansky, S., Younis, R., Liu, X., Banker, T.P., Dubovy, S.R., Langer, S.M., Berrocal, A.M. (2018). First Locally Acquired Congenital Zika Syndrome Case in the United States: Neonatal Clinical Manifestations. Ophthalmic Surg Lasers Imaging Retina. 49: e93-e98. doi: 10.3928/23258160-20180907-14.

Mittal R, Nguyen D, Debs LH, Patel AP, Liu G, Jhaveri VM, Kay S-IS, Mittal J, Bandstra ES, Younis RT, Chapagain P, Jayaweera DT and Liu XZ. (2017). Zika Virus: An Emerging Global Health Threat. Front. Cell. Infect. Microbiol. 7:486. doi: 10.3389/fcimb.2017.00486.

Mittal R, Fifer RC, Liu XZ. (2018). A Possible Association Between Hearing Loss and Zika Virus Infections. JAMA Otolaryngol Head Neck Surg. 144(1):3–4. doi:10.1001/jamaoto. 2017.1798.

Shiu C, Starker R, Kwal J, et al. (2018). Zika Virus Testing and Outcomes during Pregnancy, Florida. USA, 2016. Emerging Infectious Diseases. 24(1):1-8. doi:10.3201/eid2401.170979.

Patents: None at the time of reporting.

27. Grant #7ZK27: Identification of the Duration of ZIKV Persistence to Guide Reproductive Health Decisions

Principal Investigator: Mario Stevenson, PhD

Organization: University of Miami

Progress Report: This study proposed to define the nature of ZIKV persistence by assessing the duration of ZIKV RNA in different bodily fluids (whole blood, plasma, serum, urine, saliva, semen and female genital secretions) obtained longitudinally from men and women with acute ZIKV infection. Furthermore, the researchers planned to exploit those samples to validate a single-step assay for detection of ZIKV RNA in unprocessed samples. The study aims were to: 1.

To determine the persistence of ZIKV in different body fluids in an acute infection cohort. 2. To assess a rapid, single-step assay for detection of ZIKV RNA.

As reproductive health decisions are time-sensitive, this application will validate a point-of-care ZIKV assay that has the potential to be used in resource-rich and resource-limited settings with a high incidence of new cases. Discidium Biosciences, a University of Miami-based diagnostic company, has developed a simple, single-step assay for the detection of ZIKV RNA. Performance of the assay on unprocessed biological samples from the acute infection cohort under Aim 1 will be assessed. These results will accelerate development of an FDA's Emergency Use Authorization designated assay for detection of ZIKV RNA in bodily fluids in which ZIKV RNA persistence is most protracted. Dr. Stevenson and his team proposed that these studies will define the persistence of ZIKV in bodily fluids and help establish reliable reproductive health guidelines. In addition, they will validate a point-of-care assay that will guide pregnancy planning in resource-rich and resource-limited settings. Assay performance using the lyophilized components was exactly comparable to that of the assay using standard components. This is an important demonstration of feasibility necessary for the assay to be utilized in remote and/or decentralized testing locations.

Follow on Funding: None at the time of reporting.

Collaborations: Work was accomplished through involvement with Emory University, Baylor College of Medicine, University of Texas, St. Louis University, and John Hopkins University. As well as the University of Sao Paulo and the Federal Research Institute in Rio de Janeiro.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

28. Grant #7ZK28: Nano-Formulations of Anti-Helminthic Drugs for Zika Therapy and Prevention

Principal Investigator: Shanta Dhar, PhD

Organization: University of Miami

Progress Report: The rapid spread of Zika virus infection across the USA is anticipated to have a direct impact on the U.S. health care system as it is known to cause microcephaly as well as a spectrum of neurologic problems including seizures in newborn babies and Guillain-Barre syndrome in adults. There is a great unmet need to develop strategies to detect Zika early, but more critically to prevent the further spread of Zika by developing treatment strategies to protect newborn babies exposed to the infection. To date, Dr. Dhar's team has focused on understanding the anti-viral effects of Fc-Ivermectin- Nanoparticles (NPs) first using an *in vitro* model followed by biodel and pharmacokinetic studies in mice and dog models to understand the bioavailability of ivermectin when using oral administration with their nano-formulations. Further, they have set the basis for the clinical trial of these nano-formulations by performing safety and toxicity studies using their canine model. The study of Fc-Ivermectin-NP in dogs was used to provide a unique opportunity to evaluate both safety and distribution before the first human dose. The results from the five experiments conducted, are extremely encouraging and this innovative nanomedicine platform for ZIKA infection looks promising for future studies.

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.

29. **Grant #7ZK29:** Cellular Targets of Zika-Encoded Proteins and Microcephaly

    Principal Investigator: Alvaro NA Monteiro, PhD

    Organization: H. Lee Moffitt Cancer Center

    Progress Report: Emerging evidence suggests that the Zika virus (ZIKV) has the potential to cause Alzheimer's disease style damage to the adult brain. Proteins encoded by viruses can bind and inactivate host cell proteins leading to dramatic biological effects such as attenuation of cell growth, gene expression dysregulation and induction of cell death. Thus, Dr. Monteiro hypothesized that ZIKV-encoded proteins specifically target proteins in neural progenitor cells leading to microcephaly; and that ZIKV variants isolated in Brazil may do so with a higher affinity. To test this hypothesis, the team completed the identification of human host proteins that interact with the ZIKV non-structural proteins. This was obtained by two independent methods: a) a panel of yeast two-hybrid screenings using all non-structural ZIKV proteins as baits against a cDNA library of human brain; b) a series of tandem-affinity purifications coupled to mass spectrometry using the ZIKV non-structural proteins as baits when expressed in human 293T cells. The non-structural ZIKV corresponded to an isolate linked to microcephaly from Brazil. Protein interactors isolated via yeast two-hybrid screens were also validated in mammalian cells. The complete merged network is being compared and integrated with a dataset of human protein interactors to Dengue virus, also a flavivirus with significant similarities with ZIKV.

    The researchers encountered two main challenges during the performance of this rapid pilot. One was due to the rapid increase in the number of ZIKV variant sequences published, making the relatively simplistic approach proposed initially (i.e. compare two strains) obsolete. They tackled this challenge by strengthening their bioinformatics analysis to identify meaningful amino acid changes to be tested in the future. The second was a technical challenge to transfect neuroprogenitor cells, even after testing a very large number of transfection reagents and lentivirus. They solved this challenge by opting to complete the studies using the alternative approach in human 293T cells. This data represents the first high quality host protein-ZIKV protein interactions obtained and will serve as the groundwork to probe the biological significance of these interactions.

    Follow on Funding: None at the time of reporting.

    Collaborations: Federal Institute of Rio de Janeiro in Brazil (Dr. Marcelo Carvalho's team including Thales Nepomuceno) and the Federal University of Rio de Janeiro in Brazil (Dr. Rafael Mesquita's team including Bianca Carneiro).

    Journals: None at the time of reporting.

    Patents: None at the time of reporting.

30. **Grant #7ZK30:** Identification of Antiviral Therapies for the Treatment of Zika Virus Using Existing Drugs

    Principal Investigator: Ashley N. Brown, PhD

Organization: University of Florida

Progress Report: There is currently no vaccine or antiviral therapy licensed for the treatment or prevention of Zika virus (ZIKV). The overall aim of this project was to not only identify antivirals for the treatment of ZIKV but to also design optimal dosing strategies for effective antiviral regimens. To accomplish their aims, Dr. Brown's research team evaluated four different drugs (ribavirin, interferon-α, favipiravir, and ivermectin), all which have demonstrated antiviral potential against ZIKV. They determined exposure-response relationships for each compound and identified effective dose(s) and dosing interval(s) for active agents as monotherapy against ZIKV. Ribavirin (RBV), interferon-alpha (IFN), favipiravir (FAV), and ivermectin were screened for antiviral activity against two ZIKV strains: PRVABC59 (2015 Puerto Rico strain) and IbH 30656 (1968 Nigeria strain) via in vitro drug assays for viral production. Active agents were evaluated in the in vitro hollow fiber infection model (HFIM) and human pharmacokinetic (PK) profiles were simulated. The researchers evaluated ZIKV replication kinetics in the HFIM system using different cell lines [HUH-7 (human liver), LNCaP (human prostate), and HeLa (human cervix)] at various multiplicities of infection.

Their results suggested that alternative dosing strategies will be warranted for patients that exhibit faster clearance of FAV. Other findings showed that FAV, IFN, and RBV do not have activity against ZIKV in LNCaP cells. This lack of effectiveness may be due to the fact that these drugs cannot penetrate inside the cell to prevent viral replication. The team plans to evaluate this hypothesis in upcoming studies. They also plan to further evaluate other hypothesizes such as FAV in the HFIM system.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Pires de Mello, C. P., Tao, X., Kim, T. H., Bulitta, J. B., Rodriquez, J. L., Pomeroy, J. J., Brown, A. N. (2018). Zika Virus Replication Is Substantially Inhibited by Novel Favipiravir and Interferon Alpha Combination Regimens. Antimicrobial Agents and Chemotherapy. 62(1):e01983-17. doi:10.1128/AAC.01983-17.

Pires de Mello, C. P., Tao, X., Kim, T. H., Vicchiarelli, M., Bulitta, J. B., Kaushik, A., Brown, A. N. (2018). Clinical Regimens of Favipiravir Inhibit Zika Virus Replication in the Hollow-Fiber Infection Model. Antimicrobial Agents and Chemotherapy. 62.9. doi:10.1128/aac.00967-18.

Patents: None at the time of reporting.

31. Grant #7ZK31: Development of a Rapid Diagnostic Assay for Zika Virus Infection

Principal Investigator: Mark E. Sharkey, PhD

Organization: University of Miami

Progress Report: The main objectives of this proposal were to develop a simple, inexpensive method of specifically detecting Zika virus (ZIKV) and apply the approach to clinical samples to define the persistence of virus in distinct biological fluids. Since ZIKV levels decline rapidly after acute infection and Dr. Sharkey's research team anticipated using small sample volumes, they focused on developing an assay based on standard polymerase chain reaction (PCR) amplification to end-point to maximize detection sensitivity. Numerous primers and fluorogenic probes were carefully designed based on ZIKV genome sequence alignments to identify regions

that were well conserved in different ZIKV isolates, but dissimilar in related viruses, such as, Dengue. Comparisons of different primer/probe sets led to the identification of a set that performed with high specificity and exceptional sensitivity.

Several methods to visualize PCR products at end-point were evaluated and the team determined that incorporation of a quenched, fluorescently-labelled ZIKV probe into the reactions allowed for the unambiguous identification of positive reactions based on visualization of fluorescence upon illumination with a blue light source. As such, this method is simple, definitive and has no requirements for expensive equipment or sophisticated operator expertise. Using ZIKV virions spiked into various fluids, the team defined the limits of detection in phosphate-buffered saline (PBS), saliva, urine, semen and whole blood. Single copy detection was possible in PBS, saliva and urine samples spiked with ZIKV virions. Although more problematic, amplification of low copy number spikes was also observed in diluted semen and whole blood up to 6% of total reaction volume. The effects of PCR additives were evaluated and some were identified that enhanced target amplification in problematic biological fluids. The proposed work has been executed in the context of developing an assay that would be user-friendly and available for use in under-resourced settings. If assay performance could be maintained after lyophilization of reaction components, product stability would be enhanced and cold storage of reagents would not be required. Lyophilized reaction components were stored at room temperature for 4 months and compared to reactions using reagents that were stored under provider's guidelines. Amplification and detection using lyophilized reaction components were comparable to results obtained with standard reactions.

As there has been no active transmission of ZIKV in Florida in 2017, recruitment of participants has been challenging. During 2016, one participant had undergone study related visits and completed the study. These samples were analyzed prior to assay optimization, but ZIKV was detectable in several biological fluids over the course of one month. Subsequent samples were negative and further analysis was suspended according to the study protocol. The lack of samples, while positive for public health, has not allowed the researchers to further study ZIKV dynamics in recently infected individuals.

Follow on Funding: None at the time of reporting.

Collaborations: This project has involved various institutions, namely: Emory University, Baylor College of Medicine, University of Texas, St. Louis University, and John Hopkins University. They also obtained IRB approval from the University of Sao Paulo, to conduct studies at the Brazilian site.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

32. Grant #7ZK32: Utilization of In Utero-Diffusion Tensor Magnetic Resonance Imaging to Evaluate Neurological Disorders Caused by Zika Virus

Principal Investigator: Ulas Bagci, PhD

Organization: University of Central Florida

Progress Report: The goal of this project was to utilize fetal magnetic resonance imaging (MRI) with diffusion tensor imaging (DTI) as an innovative and transformative screening test in ZIKV infected patients to assess for potential subclinical neurological disorders in the developing fetus.

Fetal MRI is considered to be a promising in vivo method to study neurological disorders, growth, and development of the brain. Fetal MRI provides three-dimensional (3D) visualization of the fetal anatomy in a way not possible with ultrasound imaging. Furthermore, diffusion tensor imaging (DTI) is shown to be effective in investigating axonal/fiber connectivity of the central nervous system and determining dynamic microstructural changes associated with brain growth. This study's approach is innovative because it is a first of a kind systematic follow-up of anatomic and pathological descriptions derived from MRI and DTI of the subjects with ZIKV infection.

Dr. Bagci's researcher team developed a novel imaging method, which can replace the current standard of diffusion weighted imaging (DWI) due to its super-fast and accuracy properties. The idea is based on new deep learning algorithm utilizing minimum number of gradient directions in DWI (at least 7) instead of 32 (current standard). Reconstruction of the images is super-fast (due to only processing 7 directions) compared to 32 and more. This allows the research staff to have a fast scanning, which is critical for imaging unborn babies because DWI is affected from babies' motion and imaging remains a very challenging problem. Thanks to this rapid project, they have developed this new method, which can be used for various diseases, and various clinical problems, and hopefully facilitating scientific discovery in different diseases as well. Although the research team were hampered by finding subjects with Zika infections, they were able to find pregnant women (control) to be scanned with MRI and DWI, and analyzed the imaging method that they discovered. Based on the preliminary data collected, the research staff plans to submit an abstract (after patent filing) for publication and seek external funding to continue their research (general scanning procedures for unborn babies and their brain analysis with non-invasive methods) and extend the research for other diseases once external funding is secured.

Follow On Funding: None at the time of reporting.

Collaborations: University of North Carolina (Dr. Yap); NIH-Zika-initiative; Oxford University (Dr. Stamatis Sotiropolous); and the team at Florida Hospital (consultant Dr. J. Williams).

Journals: None at the time of reporting.

Patents: None at the time of reporting.

33. Grant #7ZK33: Point-of-Care Diagnostic Platform for Zika Virus Infection Based on Visual Split Deoxyribozyme Sensors

Principal Investigator: Yulia Gerasimova, PhD

Organization: University of Central Florida

Progress Report: Rapid, accurate, sensitive, and low-cost tests for Zika virus (ZIKV) infection are needed to enable efficient case management and implementation of infection control programs. The World Health Organization (WHO) recommends confirming the presence of ZIKV by National Analytical Aptitude Test (NAAT), such as reverse transcription-polymerase chain reaction (RT-PCR), using whole blood, serum, plasma or saliva samples during the acute infection stage (<7 days). The presence of viral RNA in urine is shown to be longer. In addition, parallel detection of dengue and chikunguya is also recommended. RT-PCR diagnostics offers the advantages of high sensitivity and is thus considered the gold standard of NAAT-based diagnostics.
The goal of this project was to develop a simple diagnostic platform for the detection of ZIKV infection with visual signal output that can be used at point-of-care settings and even at home. To date, a colorimetric nucleic acid based test for label-free pathogen detection has been developed

and used for the detection of ZIKV. The test relies on nucleic acid sequence based amplification (NASBA) of a viral RNA followed by interrogation of the amplicon by a cascade of deoxyribozymes constituting a visual split deoxyribozyme (vsDz) probe. The probe consists of a split phosphodiesterase deoxyribozyme, which forms its catalytic core upon binding to a specific amplicon fragment. The catalytically active complex recognizes and cleaves an inhibited peroxidase-like deoxyribozyme (PDz), thereby activating it. Active PDz catalyzes hydrogen peroxide-mediated oxidation of a colorless substrate into a colored product, thereby generating a visible signal. Viral RNA (106 copies/mL or higher) triggers intense color within two hours. The test selectively differentiates between Zika and closely related Dengue and West Nile viruses.

Follow On Funding: None at the time of reporting.

Collaborations: Scripps Research Institute (Dr. Hyeryun Choe).

Journals: None at the time of reporting.

Patents: None at the time of reporting.

34. Grant #7ZK34: Rapid Identification of Natural Products with Antiviral Activity Against Zika Virus

Principal Investigator: Michael N. Teng, PhD

Organization: University of South Florida

Progress Report: Zika virus (ZIKV) is spread by Aedes aegypti, which is also the mosquito vector for Dengue and Yellow Fever viruses. In addition to vector-mediated transfer, ZIKV has been documented to be transmitted by sexual contact. It is estimated that 80% of ZIKV infections are asymptomatic; however, ZIKV has been associated with significant neurological defects, such as Guillain-Barre Syndrome and the newly described Congenital Zika Syndrome (CZS). CZS encompasses a wide range of neurological abnormalities associated with acquisition of ZIKV infection during pregnancy. The long-term implications of ZIKV infection are not known. There is an urgent need to develop antiviral therapies against ZIKV to respond to this threat. Vaccine candidates have been rapidly advanced using currently available platforms. It is apparent that ZIKV can persist in immunologically privileged sites for extended periods of time. Thus, effective antiviral therapies will be necessary to ensure complete clearance of ZIKV from infected individuals. Development of ZIKV-specific therapies is essential. The research project proposed to develop novel assays to allow for high throughput screening of compounds to identify potential antiviral drugs against ZIKV. These assays do not require the use of live ZIKV and therefore are easily scalable and adaptable for drug discovery without the need for enhanced biosafety level protections. Primarily focusing on experiment 1, Dr. Teng and his team were able to establish a Vero cell line expressing ZIKV replicon. The expression of the replicon in the Vero-ZIKV cell line was found to be unstable. Propagation of the cell line over multiple passages resulted in loss of the replicon and resistance to drug selection. Additional Vero-ZIKV cell lines were generated and are undergoing selection. Also, in vitro transcribed replicon RNA was transfected into A549 cells but drug-resistant cell lines were not derived. Examining the antiviral screening assay, they were able to distinguish antiviral effects of the natural compounds from cytotoxic/cytostatic effects on the infected cells. This information will help screen out false positives from the list of potential hits. Compared to the previous results with the respiratory syncytial virus assay, the team has been able to eliminate several false positives. This new process will allow for more streamlined identification of hits from their ZIKV-replicon assay.

Follow On Funding: None at the time of reporting.

Collaborations: University of South Florida (Dr. Michael Teng and Dr. Bill Baker); University of Miami Miller School of Medicine (Ms. Samantha Langer, second year medical student); University of Washington in St. Louis (Ms. Kristen Smalling).

Journals: Casale, T.B., Teng, M.N., Morano, J.P., Unnasch, T., Lockwood, C.J. (2018). Zika Virus: An Emerging Infectious Disease with Serious Perinatal and Neurologic Complications. J. Allergy Clin. Immunol. 141(2): 482-491. doi: 10.1016/j.jaci.2017.11.029.

Patents: None at the time of reporting.