Biomedical Research Grant Advisory Board

Bankhead-Coley Cancer Research Program
James and Esther King Biomedical Research Program
Zika Research Grant Initiative

2016-2017 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 215.5602, Florida Statutes) and the James and Esther King Biomedical Research (King) Program (section 381.922, Florida Statutes). In fiscal year (FY) 2016-2017, 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 2016-2017, the Legislature appropriated $17,139,397, which funded a total of 18 Bankhead-Coley and King grants.

On September 22, 2016, Governor Rick Scott authorized $25 million in state funds to support the Zika Research Grant Initiative (hereafter referred to as the Zika Initiative). The intent of these research funds was to initiate new research and discoveries, with a focus on research designed to yield tangible results within three years of award. Awards from the Zika Initiative followed the same competitive peer review process as the Bankhead-Coley and King Programs. In FY 2016-2017 the Zika Initiative awarded 34 research grants.

Per statute requirements, a 2016-2017 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 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

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 16/17 funding cycle awards were made to support the following research priorities for Bankhead-Coley and King grants:

7 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: lung, breast, prostate, colon, or melanoma.

1 Award – 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.

1 Award – Tobacco Use: Reduction of tobacco use in children, adolescents, and adults.

2 Awards – 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.

1 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.

6 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).

Fiscal Year 16/17 funding cycle awards were made to support the following research priorities for Zika research:

11 Awards - Health Effects of Zika Virus: Expand upon research that improves scientific understanding of causes and subsequent impact of Zika-related morbidities in other systems (e.g., cardiovascular, pulmonary, endocrine, lymphatic, central nervous system, reproductive, developmental).

19 Awards - Innovative Diagnostic Testing or Therapeutics: Research in this area will develop methods that will determine the presence of disease in an individual suspected of having Zika.

4 Awards - Support Development of Vaccine or Other Methods: By partnering with investigators already in the process of developing a Zika vaccine, Florida research centers can establish additional clinical trial sites and increase the volume of participants.

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

For the past two 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 2016-2017.

National Institutes of Health Biomedical Research
State Funding and Rankings Fiscal Year 2016

State	Total Funding	Rank
CA	$ 3,686,026,589.00	1
MA	$ 2,572,549,176.00	2
NY	$ 2,205,949,608.00	3
PA	$ 1,570,151,520.00	4
MD	$ 1,465,624,060.00	5
NC	$ 1,154,347,750.00	6
TX	$ 1,097,661,190.00	7
WA	$ 952,837,210.00	8
IL	$ 818,027,921.00	9
OH	$ 734,159,508.00	10
MI	$ 669,562,129.00	11
FL	$ 531,720,813.00	12
GA	$ 520,595,434.00	13
MN	$ 520,225,717.00	14
TN	$ 512,414,823.00	15
CT	$ 510,609,681.00	16
MO	$ 508,984,218.00	17
WI	$ 421,776,499.00	18
CO	$ 349,974,172.00	19
VA	$ 349,479,689.00	20

Figure 1: NIH Research Funding from the 2016 Fiscal Year Reporting Period. The top twenty states in NIH funding is displayed. With over $531 million in NIH funding, Florida is ranked 12th in the nation. *Source: NIH Research Portfolio Online Reporting Tools (RePORT)*

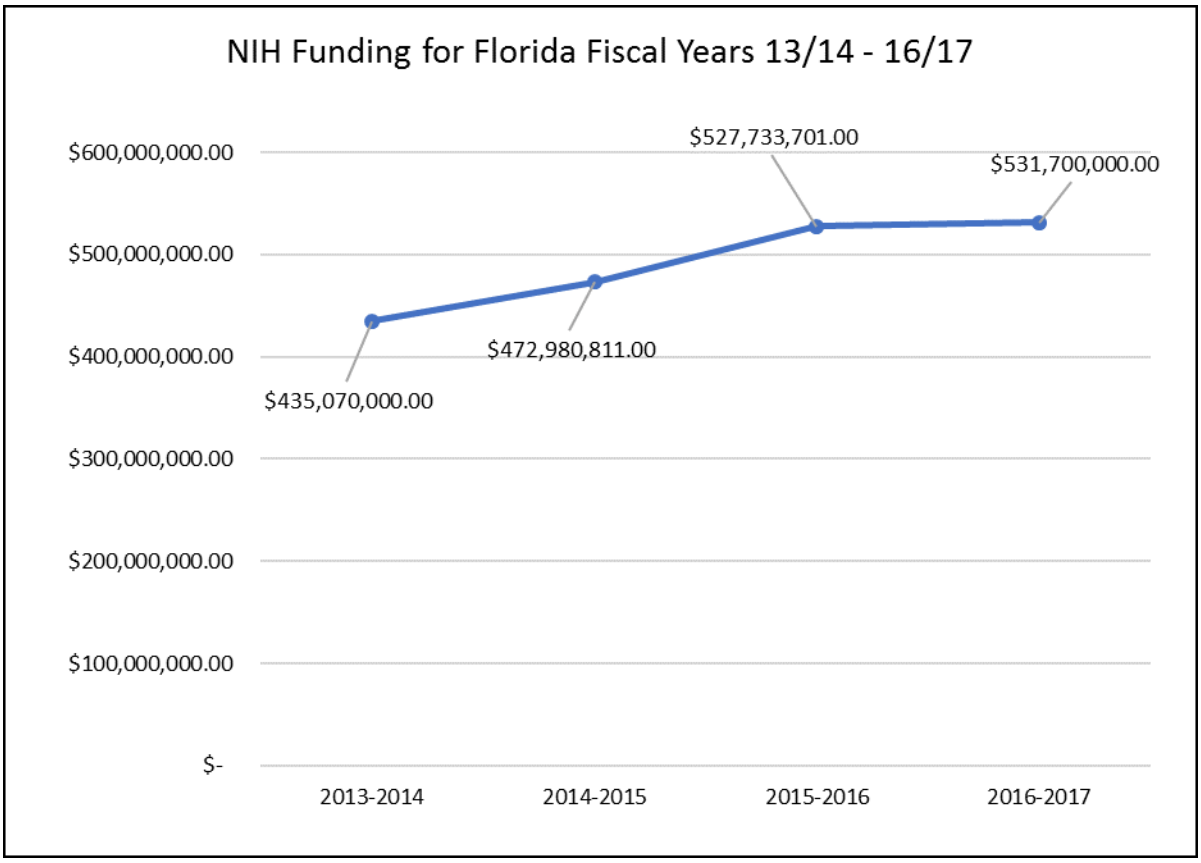

Figure 2: NIH Funding for Florida has increased in the last four years. These results reflect the Florida's initiative to expand upon research to improve scientific understanding of various diseases and health disparities.

Bankhead-Coley Cancer Biomedical Research Program

APPENDIX A

FISCAL YEAR 2016-2017 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
7BC01	University of Miami	Capobianco, Anthony	$ 1,471,318	$ 83,472.20	$ 1,387,845.80	3/17/2017	2/29/2020	No	No	No
7BC02	University of Florida	Judge, Andrew	$ 1,226,836	$ 81,506.38	$ 1,145,329.62	3/22/2017	2/29/2020	No	No	No
7BC03	University of Miami	Thomas, Emmanuel	$ 1,866,436	$ 60,172.32	$ 1,806,263.68	3/17/2017	2/28/2022	No	Yes	No
7BC04	H. Lee Moffitt Cancer Center	Gwede, Clement K.	$ 828,125	$ 21,768.99	$ 806,356.01	3/15/2017	2/29/2020	No	No	No
7BC05	H. Lee Moffitt Cancer Center	Smalley, Keiran	$ 1,468,200	$ 17,826.45	$ 1,450,373.55	4/12/2017	2/29/2020	No	No	No
7BC06	Florida Atlantic University	Wright, Amy E.	$ 622,683	$ 31,348.95	$ 591,334.05	3/27/2017	2/29/2020	No	No	No
7BC07	University of Miami	Pei, Xin-Hai	$ 97,880	$ 22,889.27	$ 74,990.73	3/08/2017	2/28/2018	No	No	No
7BC08	H. Lee Moffitt Cancer Center	Pilon-Thomas, Shari	$ 976,620	$ 59,893.73	$ 916,726.27	3/08/2017	2/29/2020	No	No	No
7BC09	H. Lee Moffitt Cancer Center	Tao, Jianguo	$ 97,880	$ 24,371.57	$ 73,508.43	3/14/2017	8/31/2017	No	Yes	No
7BC10	University of Miami	Wang, Gaofeng	$ 97,880	$ 40,358.68	$ 57,521.32	3/01/2017	8/31/2017	No	No	No
7BC11	University of South Florida	Tyson, Dinorah Martinez	$ 100,000	$ 0.00	$ 100,000	6/15/2017	6/30/2018	No	No	No

NEWLY AWARDED GRANTS FISCAL YEAR 2016-2017

(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 Capobianco, PhD

   Organization: University of Miami

   Abstract of Proposed Research: Aberrant Notch signaling is linked to many human cancers. Notch signaling has been demonstrated to play a vital role in the initiation and maintenance of the neoplastic phenotype as well as in cancer stem cell self-renewal, which may underlie a role in metastasis and resistance to chemotherapy. In this regard, Notch has become an exceedingly attractive therapeutic target in cancer. However, full range of potential inhibitors targeting the pathway has not been well explored. There is a great interest in designing small molecule inhibitors to directly target the Notch transcription complex, either by blocking the assembly of Notch transcriptional activation complex or by inhibiting the activation of the Notch pathway. Previously, the research team reported the identification and characterization of Notch activation complex kinase (NACK), which acts as a Notch transcriptional co-activator and an essential regulator of Notch-mediated tumorigenesis and development. Given the critical role of NACK in Notch pathway, they hypothesize small molecule inhibitors of NACK activity will function as specific Notch transcriptional activity inhibitors, and therefore be effective as anti-neoplastic agents for Notch-dependent tumors. The overall goal of this project is to develop and validate additional lead candidates from the scaffold of the lead compound (iNACK, Z271-0326) to develop novel potent drug-like small molecule inhibitors of NACK as clinical candidates. Successful completion will provide specific and direct inhibition of the Notch transcriptional activation complex, which will open avenues for the development of new therapies for the Notch-dependent cancers. The project's three specific aims are: (1) Lead optimization of NACK inhibitor Z271-0326 by iterative computational design and chemical synthesis of the predicted best compounds, (2) Identification and validation of lead analogs of iNACK through biochemical and biological assessment, (3) Preclinical evaluation of lead clinical candidates.

   Follow On Funding: None at the time of reporting.

   Collaborations: Dr. Rhett Kovall, University of Cincinnati, College of Medicine, Dr. Kovall's group is working to elucidate the crystal structure of NACK-inhibitor complexes. Currently, one graduate student is receiving training under the direction of Dr. Kovall

   Journals: None at the time of reporting.

   Patents: None at the time of reporting.

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

   Principal Investigator: Andrew Judge, PhD

   Organization: University of Florida

Abstract of Proposed Research: Cachexia is a devastating condition that affects up to 80% of cancer patients and is characterized and defined by progressive skeletal muscle wasting and body weight loss. This loss of muscle mass is associated with reduced tolerance to chemotherapy and increased complications from surgical and radiotherapeutic treatments. Consequently, cachexia decreases survival time in cancer patients and cachexia itself is estimated to be responsible for up to 30% of all cancer-related deaths. Unfortunately, there are currently no medical therapies to counter cancer-induced muscle wasting which is due, in part, to a lack of understanding of the initiating mechanisms. This proposal was developed to identify novel mechanisms which initiate limb and respiratory muscle wasting in response to cancers of the lung, colon and pancreas, which is critical to the development of therapeutic strategies to enhance the quality of life and survival of cancer patients. Specifically, this proposal will focus on the role that two specific proteins, called interleukin 8 and CXCL1 (a chemokine ligand 1 motif), play in the initiation of cancer-induced muscle wasting. Both of these proteins are increased in the serum of cancer patients and their receptors are increased in the muscle of cancer patients. The biological importance of these proteins and their receptors will be studies as they relate to cancer cachexia.

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 HCC

   Principal Investigator: Emmanuel Thomas, MD, PhD

   Organization: University of Miami

   Abstract of Proposed Research: Hepatocellular carcinoma often occurs in the setting of liver disease. This cancer is frequently diagnosed in the later stages and it has a median survival of 6-20 months. Chronic hepatitis C virus infection is a primary risk factor for the development of hepatocellular carcinoma. Hepatocellular carcinoma is one of the few cancers whose incidence is increasing in the United States mainly due to the aging Hepatitis C infected population. African Americans have the highest disease prevalence of Hepatitis C among all the racial groups in the United States. Latinos in the United States also have a higher disease prevalence of Hepatitis C and a near double Hepatitis C-related mortality rate when compared to non-Latino whites. Furthermore, Hepatitis C infection in Latinos is typified by more severe inflammatory activity and fibrosis, a larger disease progression, and a higher risk of cirrhosis. Because the genotype leading to better response is in substantially greater frequency in European than African populations, this genetic polymorphism can explain much of the difference in incidence rates between them and other racial groups. This proposal will undertake a longitudinal prospective study of 1,000 community residents, 500 with Hepatitis C virus infection, with 4 waves of follow-up assessment after the baseline assessment. The repeated standardized ultrasound, used for Hepatocellular carcinoma surveillance and screening, and other assays will make it possible to study progression of clinical and lab-assessed concomitants of liver disease and Hepatocellular carcinoma development as well as discrete outcomes (e.g., liver transplant) expressed as a function of standard covariates (e.g., sex, age, body mass index, serum

markers of liver disease) plus known and implicated genetic influences (e.g. interleukin 28B genotype). Ultimately, the study will probe into the possible interactions of Hepatitis C infection and hepatocellular carcinoma with race-ethnicity variations and obesity, shedding new light on these minority research challenges.

Follow On Funding: None at the time of reporting.

Collaborations: This project is currently providing training to two students: a second-year medical student and a second-year graduate student.

Journals: Thomas, E. Hepatitis B virus X protein: TRIMming antiviral defences in hepatocytes. Gut. 2017 May 5. doi:10.1136/gutjnl-2017-314013.

Feldman, E.B., Balise, R., Schiff, E., Whitehead, N., Thomas, E. Barriers to Hepatitis C screening in a minority population: A comparison of Hepatitis C and human immunodeficiency virus screening rates at a community STD clinic in Miami, Florida. J. Community Health 2017. 42 (5): 921-925

Patents: None at the time of reporting.

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

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

   Organization: H. Lee Moffitt Cancer Center

   Abstract of Proposed Research: A leading cause of death in the U.S. is colorectal cancer (CRC). It is a significant health concern that affects both men and women and many adults do not get screened. Community involvement is needed for sustainable solutions. The proposed study called Community CARES (Colorectal Cancer Awareness, Research, Education and Screening) or C-CARES for short, tests a promising intervention delivered in Federally Qualified Health Centers (FQHCs). The investigative team recently completed an intervention study in clinics called CARES that was guided by community members, and which tested low-literacy materials (i.e., photonovella+DVD) + FIT. In this study, 80% of participants got screened with FIT, a rate that exceeds Healthy People 2020 CRC screening goal of 70.5% and the national goal to reach 80% by 2018. Although highly beneficial, the CARES study emphasized initial vs. repeat annual screening behaviors to help increase effectiveness of FIT. C-CARES extends this foundational work by collaborating with community clinics. It seeks to implement a multicomponent, dual-language (English/Spanish), theory-driven educational intervention to promote long-term annual screening with FIT. In Phase I - the Preparatory Phase (months 0-6), the team activates its Community Advisory Board, completes packaging of additional C-CARES components, and finalizes procedures to utilize existing electronic medical record systems at the FQHCs—an important tool for identifying eligible patients for screening, delivering patient reminders, and documenting CRC screening completion. In Phase II - the Intervention Phase (months 7-60), a two-arm randomized comparative design will be used to examine whether C-CARES Plus versus C-CARES improves annual FIT screening among 328 individuals, 50-75 years of age, who are not up to date with CRC screening. In the C-CARES group, participants are given CARES materials + FIT kit. In the C-CARES Plus group, a stepped approach is used: participants are given CARES materials + FIT kit plus added personalized components that include one-on-one education, mailed or text message reminders, and education. It is expected

that C-CARES Plus will result in greater screening rates at 3, 15, and 27 months. This sets the stage for future statewide dissemination for improved community health. The study will also help to impact health disparities in colorectal cancer.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

   Principal Investigator: Keiran Smalley, PhD

   Organization: H. Lee Moffitt Cancer Center

   Abstract of Proposed Research: Uveal melanoma is the most common primary cancer of the eye. It arises from melanocytes that reside in the uveal tract of the eye and tends to be most common in individuals who are at risk for skin melanoma (e.g. blue eyes, blonde hair). Although most patients with uveal melanoma present with local disease only, half will eventually succumb to distant metastases – even when the primary tumor is treated successfully. At this time, there are no effective treatments for disseminated uveal melanoma, and even treatments that have proven effective for skin melanoma such as immunotherapy seem ineffective in uveal melanoma. It is possible to convert the high-risk subset of tumors to low risk through use of drugs that regulate tumor cell plasticity called HDAC (histone deacetylase) inhibitors. Specific HDAC inhibitors can sensitize uveal melanoma cells to other experimental drugs that are being evaluated in the clinic, such as methyl ethyl ketone (MEK). The goal of this proposal is to determine the mechanisms that push some uveal melanomas into the high-risk category and to characterize whether this presents new therapeutic opportunities. Ultimately, new therapies can be developed that target high risk uveal melanoma with the expectation of evaluating these clinically in the near future. This proposal represents a collaborative effort between three investigators with extensive expertise in uveal melanoma (Dr. Harbour), signaling and therapy (Dr. Smalley) and tumor cell plasticity (Dr. Licht).

   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 laboratories.

   Journals: None at the time of reporting.

   Patents: None at the time of reporting.

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

   Principal Investigator: Amy E. Wright, PhD

   Organization: Florida Atlantic University

   Abstract of Proposed Research: Natural products also called “genetically encoded small molecules” and their derivatives have long been important in medicine. Natural products

represent over 48% of the clinically approved cancer chemotherapeutics. Compounds such as paclitaxel, adriamycin, irinotecan and the vinca alkaloids are all important cancer drugs used clinically every day. Many have complex three dimensional structures that allow them to interact directly with biologically important macro-molecules such as RNA and DNA (ribonucleic acid and deoxyribonucleic acid). This project seeks to investigate a library of natural products derived from marine plants and invertebrates for their ability to reduce levels of a protein called survivin. Survivin is typically not observed in normal tissues but is present in large amounts ("upregulated") in cancer tissues. It is considered to be a "nodal protein" for cancer because it has important roles in many cancer related processes. Survivin levels correlate with poor prognosis for a number of cancers including colon, lung and breast cancer. This research project proposes to discover and carefully investigate the biological responses of new chemical structures (natural products) that reduce levels of survivin in colon, lung and breast cancer cell lines. The Harbor Branch Oceanographic Institute of Florida Atlantic University library of natural products will be used in this study and has over 18,000 specimens, many of them from unusual deep-water habitats. Past work with the library has found one compound isolated from a Florida sea squirt that is approved for the treatment of soft tissue sarcoma and ovarian cancer. Other compounds are under investigation for a variety of uses including cancer, tuberculosis, malaria and Alzheimer's disease. The active molecules will be purified, their structures defined and in-depth biological characterization will be conducted. Compounds found in this way will be moved forward for translation into effective new cancer selective chemotherapeutics.

Follow On Funding: None at the time of reporting.

Collaborations: One doctoral student at Florida Atlantic University

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

   Principal Investigator: Xin-Hai Pei, MD, PhD

   Organization: University of Miami

   Abstract of Proposed Research: Basal-like breast cancers (BLBCs) are the most lethal breast cancers, partly due to their enrichment with cancer stem cells (CSCs) are thought to drive relapse and metastasis. More than one third of BLBCs have dysfunctional breast cancer susceptibility genes (BRCA1). Although the majority of BRCA1 deficient cancer patients respond to DNA-damaging agents, tumor recurrence and resistance combine to decrease the survival. Thus, additional therapies targeting the pathways aberrantly activated by BRCA1 deficiency are urgently needed. Protein kinase C (PKC) and cyclin-dependent kinase 6 (CDK6) are major kinases activated in CSCs and BLBCs. Activation of platelet-derived growth factor receptor (PDGFR) signaling results in the PKC-dependent activation of Fos-related antigen-1 (FRA1), thereby leading to the assembly of FRA1-c-JUN (Fos-related antigen-1 Jun Proto-Oncogene) complexes, activation of epithelial-mesenchymal transition (EMT) program, and generation of CSCs. The research staff discovered that deletion of p16Ink4a protein (p16) or p18Ink4c protein (p18) inhibitors of cyclin-dependent kinase 4 and 6 (CDK4 and CDK6, respectively) in mice led to mammary cell proliferation. Disrupting BRCA1 in p16- or p18-deficient mice activated EMT, which is associated with CSC expansion and BLBC development. More p18; BRCA1 or p16; BRCA1 double mutant tumors expressed higher levels of PDGFR,

phosphorylated-PKC, and FRA1 than p18 single mutant tumors. Inhibition of PDGFR or PKC activity reversed EMT in BRCA1 deficient tumor cells. They hypothesize that BRCA1 suppresses PDGFR-PKC-FRA1 signaling and collaborates with the INK4-CDK6 (inhibitor of cyclin-dependent kinase 4 bonded to kinase 6) in the pathway to control CSCs and BLBCs. They propose two aims to test this: Aim 1: determine the role of PDGFR-PKC-FRA1 signaling in BRCA1 mediated tumor suppression, and Aim 2. determine whether PDFGR-PKC-FRA1 signaling collaborates with CDK6 to drive BRCA1 deficient CSCs and how BRCA1 regulates the assembly of FRA1-c-JUN complex. This proposal has two impacts: 1. Identifying PDGFR-PKC-FRA1 signaling as a downstream signaling of BRCA1 in the suppression of CSCs provides a new pathway to be therapeutically targeted for BRCA1 deficient tumors. 2. Discovering that CDK6 cooperates with PDGFR-PKC-FRA1 signaling to control CSCs allows two pathways to be targeted in BLBCs.

Follow On Funding: None at the time of reporting.

Collaborations: In collaboration with Mr. Cheng Fan, a Bioinformatics Specialist at the University of North Carolina at Chapel Hill to develop statistical strategies and biostatistical analyses for the experiments proposed. In collaboration with Dr. Anthony Capobianco, a University of Miami expert in the investigation of Notch transcriptional regulatory complex in cell differentiation and cancer development, to study the activating protein 1 (AP-1) complex. In collaboration with Dr. Tan Ince, a University of Miami pathologist, to study tumor initiation and metastasis.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

8. **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

   Abstract of Proposed Research: 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 melanoma cancer patients 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 to use adoptive cell therapy (ACT) on 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. 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. In addition, suppressive factors including MDSC are reduced during lymphopenia, allowing for maximum activity of transferred T cells. Lymphopenia is a temporary state. This rapid repopulation of highly suppressive MDSC may decrease the effectiveness of ACT by shutting off T cells and preventing complete tumor regressions. The research proposed in this

application will improve the understanding of MDSC expansion and suppressive functions in the setting of lymphopenia and determine the effects of blocking MDSC expansion and function on T cell responses after ACT. The goals of this proposed research are threefold: 1. To evaluate the role of MDSC populations at the tumor site after induction of lymphopenia; 2. To define the factors that contribute to the rapid expansion and function of MDSC populations in the setting of lymphopenia; and 3. To examine the reconstitution and function of MDSC populations in melanoma patients enrolled in ongoing ACT clinical trials. Achievement of these goals will determine whether eliminating MDSC in the setting of lymphopenia is feasible to improve the activity of tumor-specific T cells. Novel approaches based on MDSC blockade and ACT may result and improve therapies for patients with 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.

9. **Grant #7BC09:** Ibrutinib Resistance Mechanism in Mantle Cell Lymphoma

   Principal Investigator: Jianguo Tao, MD, PhD

   Organization: H. Lee Moffitt Cancer Center

   Abstract of Proposed Research: There are many types of cancers in the human body. Moffitt research staff are specifically focusing their study on lymphoma. Most cancer patients with lymphoma are put on drugs called chemotherapy to help destroy cancer cells. For some patients, chemotherapy is not helping them because the lymphoma cells become drug resistant. Mantle cell lymphoma (MCL) is a cancer that arises in lymphoid organs and an aggressive type of lymphoma. This type of lymphoma is often associated with an adverse prognosis, aggressive clinical fatal course and shortened survival due to drug resistance. There is evidence that demonstrates the interactions between cancer cells and the surrounding cells near them. Dr. Tao and staff believe that these surrounding cells in the environment; play an important part in how lymphoma cells develop and respond to chemotherapy and acquire secondary drug resistance. This study is designed to discover, how the surrounding environment affects the cancer cell growth and how lymphoma (MCL) responds to chemotherapy, so they can help lymphoma patients who are not usually being helped by chemotherapy.

   Follow On Funding: None at the time of reporting.

   Collaborations: 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. Unification of de novo and acquired ibrutinib resistance in mantle cell lymphoma. Nat. Commun. 2017 8:14920. doi:10.1038/ncomms14920

   Patents: None at the time of reporting.

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

Principal Investigator: Gaofeng Wang, PhD

Organization: University of Miami

Abstract of Proposed Research: Breast cancer is one common malignancy that predominantly affects women. The onset of breast cancer results from a combination of genetic and environmental risk factors, such as certain life styles and diets. The laboratory staff recently found that vitamin C, a micronutrient, is essential for ten-eleven translocation (TET) enzymes to generate a special component named 5-Hydroxymethylcytosine (5hmC) in DNA. In many types of cancers, including breast cancer, 5hmC has been found at either a very low level or undetectable. The loss of 5hmC changes the function of many genes, which contribute to the transformation of healthy breast cells into cancerous breast cells. Previous studies have shown that increasing the amount of the TET enzymes in breast cancer decreases its malignancy. While increasing TET level in patients might not be clinically feasible, finding a means to therapeutically restore normal 5hmC content may ultimately help reverse the malignant phenotype and yield a novel therapy for breast cancer. Vitamin C appears to conveniently restore 5hmC in the cell. Treating breast cancer cells with vitamin C resulted in decreased invasiveness, inhibited cell growth along with an elevation of 5hmC content. Based on these promising results, this research proposes to test whether vitamin C can prevent the onset and progression of breast cancer. If they can successfully reduce the malignancy of breast cancer in rodent models by repletion of vitamin C, this would support a similar therapeutic approach in breast cancer patients to delay or prevent disease progression.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

11. **Grant #7BC11:** Women at Work: Multi-ethnic Comparison of Cancer Survivors with Low-status Jobs

Principal Investigator: Dinorah Martinez Tyson, PhD, MPH, MA

Organization: University of South Florida

Abstract of Proposed Research: There is limited knowledge about work outcomes among female cancer survivors who were employed in low-status occupations at time of diagnosis. The research team seeks to understand the underlying work environments that exacerbate health disparities and affect cancer morbidity, particularly among minority cancer survivors. This exploratory, developmental project stems from a strong partnership between academic team members at the Tampa Bay Cancer Community Network (TBCCN) and two community-based organizations: Faces of Courage and Latinos Unidos por un Nuevo Amanecer, Inc. (LUNA). The study aims to: 1) Explore work-related decisions and the challenges experienced by cancer survivors who work in low-status occupations and 2) Develop a survivor and employer advisory board that will inform the research process and provide input that will strengthen the National Institutes of Health proposal re-submission. The proposed qualitative study will advance knowledge about work environment, social context, and cultural factors that influence work-related challenges and employment status following cancer treatment among female cancer survivors in low-status occupations, with a focus on minorities. They will conduct interviews with

a total of 30 women who were working in low-status jobs at the time of cancer diagnosis. They will include an equal number of Hispanic, African American and European American cancer survivors. This will enable them to gain preliminary knowledge about post-diagnosis work experiences of diverse cancer survivors, and minorities. Findings will inform broader employment policies and procedures, and implications and application for future psycho-educational interventions and work-related training programs that engage and support cancer survivors.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Bankhead-Coley Cancer Biomedical Research Program

APPENDIX B

FISCAL YEAR 2016-2017 ACTIVE GRANTS,
Funding Year 2015-2016

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
6BC02	University of Miami	Capobianco, Anthony	$ 1,343,732.02	$ 390,498.42	$ 953,233.60	4/20/2016	3/31/2019	No	No	No
6BC04	University of Florida	Tran, David D.	$ 1,784,753.25	$ 479,919.93	$ 1,304,833.32	3/04/2016	3/31/2021	Yes	No	No
6BC05	Mayo Clinic Jacksonville	Thompson, Aubrey	$ 1,064,624.44	$ 428,755.61	$ 635,868.83	4/12/2016	3/31/2019	No	Yes	No
6BC06	University of Miami	Antoni, Michael	$ 1,784,945.19	$ 304,970.19	$ 1,479,975	4/15/2016	3/31/2021	No	No	No
6BC07	University of Florida	Kladde, Michael	$ 681,887.44	$ 102,447.52	$ 579,439.92	3/31/2016	3/31/2019	Yes	No	No
6BC08	H. Lee Moffitt Cancer Center	Mahajan, Nupam	$ 1,329,860.86	$ 267,659.36	$ 1,062,201.50	4/15/2016	3/31/2019	No	Yes	No
6BC09	University of Florida	O'Dell, Walter	$ 1,445,736.61	$ 287,099.16	$ 1,158,637.45	3/19/2016	3/31/2021	Yes	Yes	No

ACTIVE GRANTS FISCAL YEAR 2016-2017

(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: A structure-activity relationship (SAR) study of NADI-260 (Notch inhibitor compound) analogs to identify more potent compounds that are less prone to metabolic degradation led to the identification of NADI-351 (Notch inhibitor compound), which exhibited a half maximal inhibitory concentration (IC_{50}) of 2.4 nM (nanometer) in the Notch-signaling transcription complex (NTC) AlphaScreen-based assay. To determine the metabolic stability of NADI-351, the research team determined the plasma pharmacokinetic profile of NADI-351 in mice. The study was performed in male C57BL/6 mice following a single intravenous and intraperitoneal dose administration at 2 and 100 mg/Kg (milligrams/Kilogram), respectively. Mean pharmacokinetic parameters were calculated using the non-compartmental analysis tool of Phoenix WinNonlin (Version 6.3). The data show that following a single intravenous administration of NADI-351 (2 mg/Kg), compound showed low plasma clearance [18.69 mL/min/Kg (milliliter/minute/Kilogram)] considering the normal liver blood flow in mice is 90 mL/min/Kg, with elimination half-life of 1.16 h. In the case of intraperitoneal administration, plasma concentrations of NADI-351 were detected up to 24 h with T_{max} (maximum concentration in serum) of 0.25 h. Pharmacokinetic data indicate that NADI-351 exhibits higher metabolic stability than NADI-260 as well as higher *in vitro* potency. Staff performed a metabolite identification of GSH-EE (glutathione reduced ethyl ester) adducts of NADI-260 and NADI-351 in human liver microsomes (in presence and absence of Nicotinamide adenine dinucleotide phosphate, NADPH) using LC-MS/MS in order to guide further structural modification and predict *in vivo* performance. The results indicate that NADI-260 showed GSH-EE conjugation [m/z (mass-to-charge) 787], whereas the glutathione adduct was not found in NADI-351 when incubated in human liver microsomes. These results suggest that adding a methyl group on the alkene position in NADI-351 (R7 position in the general scaffold) might be responsible for blocking the site of glutathione conjugation, therefore turning off Michael acceptor activity while improving *in vitro* potency. Together, the pharmacokinetic and glutathione reactivity data indicate an improved stability of NADI-351 over NADI-260 and the next steps are to evaluate the effect of NADI-351 in Notch dependent and independent cell lines, evaluation of biochemical properties as well as studies in mouse xenografts to determine the effect of NADI-351 on tumorigenesis. In addition, they have continued exploring structure-relationship studies of NADI-351 analogs to identify analogs with similar properties, but with structural substituents that may explore additional chemical space in the NTC. They are performing these studies by rational design of analogs using molecular docking simulations of potential compounds using the structure of the NTC as the receptor (MOE software) in combination with the *in vitro* NTC AlphaScreen-based assay. These studies are underway.

   Follow On Funding: None at the time of reporting.

   Collaborations: Dr. Rhett Kovall, University of Cincinnati, College of Medicine. One graduate student is receiving training under the direction of Dr. Kovall.

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: Specific aims I and II: To find a dose in Phase 1 using interleukin 6 receptor (IL-6/R) inhibitors plus Carboplatin in Metastatic Breast Cancer and a Randomized, Open-Labeled Phase 2. Study of IL-6/R inhibitor plus Carboplatin vs. Carboplatin Alone in Stage II-III Triple Negative Breast Cancer. After submitting their initial protocol to the Scientific Review and Monitoring Committee (SRMC) at the University of Florida Cancer Center in late May 2017, the SRMC recommended the following two main changes: A) the SRMC requested that they change their choice of chemotherapy drug from carboplatin to capecitabine. Once low proliferative disseminated tumor cells (lpDTCs) are re-awakened, in theory the cells then become sensitive to most, if not all, cytotoxic drugs. Data support the use of paclitaxel and carboplatin, two cytotoxic drugs with different mechanisms of action, thus consistent with the notion that there is a class effect of cytotoxic drugs in the disseminated tumor cell (DTC) elimination approach. Therefore, a switch to capecitabine should not affect the scientific validity of the study. To confirm that indeed there is a class effect of cytotoxic drug, they performed an experiment similar to that reported in the original proposal, except that bone marrow-derived basal-like breast cancer cells (MDA-MB-231), a human triple negative breast cancer cell line, was used. And instead of arresting cells by class A basic helix-loop-helix protein 38 or Twist-related protein 1 (Twist1) expression, they simply treated these cells with transient transforming growth factor beta 1 (TGFb-1), to force them into dormancy or delayed growth arrest. Cells were then either left untreated or treated with 2µg/ml (micrograms/milliliter) anti-IL-6R goat polyclonal antibody (R&D) or goat Immunoglobulin G (IgG) control followed by Fluorouracil (or 5-FU, the main cytotoxic metabolite of capecitabine). Pretreatment with the IL-6R neutralizing antibody greatly increased sensitivity to 5-FU compared to the control IgG [IC50 of 14.8 µM (micromoles) vs. >80 µM, respectively], thus confirming that capecitabine has similar efficacy as other chemotherapy drugs tested. With these data, they proceeded with making the switch from carboplatin to capecitabine. The standard regimen for capecitabine for North American patients is 1000mg/m2 (milligrams/square meter) Per os (PO) daily, 2 weeks on, 1 week off, for 8 cycles. B) Since the Twist1-Snail1 (Zinc finger protein SNAI1) ratio test is not certified by the Clinical Laboratory Improvement Amendments (CLIA), the SRMC suggested that it should be used for stratification and analysis and not for screening potential subjects, as a CLIA certified lab would be required in that instance. This requires changes to the design of the phase 2. They demonstrated that interleukin 6 (IL-6) is produced directly by lpDTCs, which through an autocrine or paracrine network activates the p38 (protein kinase) pathway specifically in these tumor cells. The more upstream role of IL-6 in the Twist1-IL-6/Rp38 axis makes it a more attractive target. However, the IL-6 centric view does not rule out a tumor cell extrinsic (tumor microenvironment – TME) or Twist1- independent mechanism of lpDTCs. Bone marrow DTCs, or BM DTCs, are sheltered in niches composed of bone marrow cells such as hematopoietic stem cells, hematopoietic progenitor cells, osteoblasts, osteoclasts, bone marrow mesenchymal stem cells etc., which provide cytokines and growth factors to DTCs and protect them from

immune detection and clearance. Therefore, both tumor cell intrinsic and extrinsic properties must be targeted to achieve success. From that standpoint, IL-6 is a much better target that will provide them both opportunities. They showed in previous reports that cell-free supernatants, collected from culture of normal epithelial or breast cancer cells that were induced to undergo epithelial-mesenchymal transition (EMT) with transient TGFb, were able to induce classical EMT changes in the same native parental breast cancer and epithelial cells, as evidenced by morphological changes, down regulation of the epithelial marker E-Cadherin and up regulation of the mesenchyme marker Valentine. These parental breast cells should lack active EMT factors because they are resting epithelial cells. They demonstrated that the secreted factor present in these supernatants was IL-6. By extension, this supports the notion that lpDTCs are a major source of IL-6 production that they themselves consume. But these data do not limit the source of IL-6 production to only these lpDTCs. IL-6 can also be produced by cell surrounding these tumor cells (e.g. in the DTC niche/TME, osteoblasts, bone marrow mesenchymal stem cells etc. are well known robust IL-6 secretors). The supernatant transfer experiment clearly showed that IL-6 alone was sufficient at inducing epithelial cancer cells (which by definition lack active EMT factors) to activate the EMT and thus dormancy program. Their interpretation is that it may be less important what the source of IL-6 secretion is (DTCs or DTC niche cells), it's more important that IL-6 is critical at inducing and maintaining EMT/lpDTCs in an autocrine/paracrine fashion. In another word, their results support the tumor cell-intrinsic view but at the same time neither do they negate the TME view. In fact, they may gain additional information by expanding selection. Next is the evidence that although Twist1-positive lpDTCs are a major source of chemo resistance, they are hardly the only one. Mice were treated the chemo Taxol. As demonstrated, Taxol decreased the number of DTCs, although not completely as expected since the remaining cells were chemo resistant (i.e. lpDTCs). When they determined the fraction of lpDTCs defined as Twist1+, p-p38+, bromodeoxyuridine-) in these remaining lpDTCs, Taxol significantly increased the relative fraction of lpDTCs as would be expected. Even more telling, however, was that in Taxol treated animals, only about 70% of chemo resistant lpDTCs were Twist1 positive. Yet when tumor mice were treated with a combination of IL-6 inhibitor and chemotherapy, the number of lpDTCs was profoundly diminished >90%, suggesting that the combination eliminated more than just Twist1-positive DTCs. Taken together these observations and others indicated that although Twist1-positive DTCs are a major source of chemo resistance, there is also a rationale for not excluding Twist1-negative DTCs as these cells may still be dependent on IL-6, which presumably is secreted by surrounding TME cells. They focused on the Twist1-positive population in the original proposal because they wanted to pick a narrow window to achieve a small sample size. But clearly Twist1-positive tumor cells are likely not the exclusive IL-6 dependent cells. About 1/3 of DTCs are Twist1 negative but still appear sensitive to IL-6 inhibition. From the scientific standpoint, either approach will produce useful data to move forward to a larger trial, but an IL-6 focus may get them there faster. The goal of aim 3 is to identify new mechanisms and targets to further improve this treatment approach. C) In addition, COR-001, Corvidia Inc.'s IL-6 inhibitor, is a specially modified monoclonal antibody that gives it a significantly long half-life (3 months!). Upon further discussion with Corvidia, it became clear that COR-001 might not be ideal for this application because it will be difficult to control the exact timing of IL-6/R inhibition in relation to cytotoxic chemotherapy. One of the risks of the proposed approach is the ability to reactivate lpDTCs. In the absence of effective control of these reactivated lpDTCs, the risk of metastasis may be increased. Therefore, the researchers have decided to use one of the Food and Drug Administration (FDA) approved IL-6/R inhibitors that has a shorter half-life and can be given

immediately prior to each cycle of capecitabine chemotherapy. Sarilumab, approved for rheumatoid arthritis, is ideal. It has a shorter half-life, is given at a fixed dose by subcutaneous injection, and has a favorable toxicity profile. In their preclinical data, only three such cycles were adequate at significantly reducing the number of DTCs and increasing survival. For the human study, they propose to add sarilumab to the first 4 cycles of capecitabine. They anticipate that since the protocol's two drugs are FDA approved, the toxicity monitoring of the combination will be easier and the FDA review and approval process will be simplified. D) With these changes, they are required to modify their statistical analysis and use a molecular endpoint as the primary endpoint and the original 2-year progression-free survival as the secondary endpoint. Clearance of BM DTCs have been shown to carry significant prognostic implication. This modification will ensure a manageable sample size and timely completion of the trial to determine whether a larger phase 3 trial will be warranted to confirm the results. Specific aim 3: To identify actionable genetic and somatic targets of lpDTCs using a combination of genomics, expression profiling and the computational inferred network platform, ARACNe. They are finalizing the reference networks for all subtypes of human breast cancer, including the triple negative subgroup. They have also optimized all the bone marrow processing protocols and quantitative polymerase chain reactions for humans. All of the original human work was performed in a previous institution and currently needs to be optimized for this proposal. With these efforts, they aim to be well prepared for the first human samples becoming available from this study.

Follow On Funding: None at the time of reporting.

Collaborations: Collaboration with Corvidia, Inc will provide the IL-6 inhibitor (COR-001) to the studies and support all the Pharmacokinetic/Pharmacodynamic studies of this drug in breast cancer patients. New collaborative projects are developing with Dr. Chenglong Li in the College of Pharmacy at University of Florida.

Journals: None at the time of reporting.

Patents: Tran, D.D. chemotherapeutic resensitization of disseminated cancer stem cells through reactivation by p38 inhibition – Chemotherapeutic Methods. International application number PCT/US2017/09240. Filed 2/24/17.

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

Principal Investigator: Aubrey Thompson, PhD

Organization: Mayo Clinic - Jacksonville

Progress Report: A novel methodology for evaluating clinical outcome data has been developed and a manuscript describing this protocol is currently under review. Genomic analysis via NanoString, has revealed gene expressions in the north central cancer treatment group N9831 samples. Analysis of Cox hazard ratios for individual genes is complete. Analysis of molecular tumor infiltrating lymphocyte signatures (mTILs) is ongoing, in collaboration with NanoString, with a view towards assigning scores to individual patients that reflect the distribution of immune cell subtypes within each N9831 sample. The preliminary data was presented at the American Association for Cancer Research (AACR) and a manuscript is in development describing the relationship between histological analysis of tumor infiltrating lymphocytes, molecular signatures associated with tumor infiltrating lymphocytes, patient age,

and outcome. A manuscript is in preparation comparing outcome as a function of treatment and immune infiltration status and age. The data indicate that there is a strong age-related association with outcome following adjuvant trastuzumab. Clinical validation of the immune enrichment score as a predictive marker of response to adjuvant trastuzumab is in early stage human epidermal growth factor receptor 2 (HER2+) patients. Ribonucleic acid (RNA) has been obtained from Finland human epidermal growth factor receptor and capecitabine trial, and the gene expression of the intrinsic subtype, immune function genes, and immune cell subtype molecular signatures have been analyzed. The team is collaborating with the National Surgical Adjuvant Breast and Bowel Project (NSABP) to evaluate intrinsic subtypes from samples collected as part of the B31 trial. This collaboration involves using the prediction analysis for microarray (PAM50) test to re-analyze the gene expression and calibration of signals from the PAM50 codeset to the Prosigna codeset. Work is being completed with collaborators at NanoString and NSABP to determine the approach to assigning intrinsic subtype using the Prosigna algorithm. Initial results indicate that the distribution of subtypes using the Prosigna algorithm is essentially identical in N9831 and B-31. A data transfer agreement was developed with Breast International Group (BIG) for obtaining RNA-sequence data and pathological complete response data from the Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (NeoALTTO) trial of trastuzumab, lapatinib, and trastuzumab plus lapatinib in the neo-adjuvant setting. Work is underway with Emerald Logic to evaluate an alternative statistical model for predicting response based on gene expression data in combination with clinical parameters.

Follow On Funding: None at the time of reporting.

Collaborations: Working with NSABP and NanoString. Collaboration is in place Kate Pogue-Geile, National Surgical Adjuvant Breast and Bowel Program and Charles M. Perous, 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. Intrinsic subtype and therapeutic response among HER2-positive breast tumors from the NCCTG (Alliance) N9831 trial. J. Nat'l Cancer Inst. 2017. 109(2): 1-8 doi:10.1093/jnci/djw207

Patents: None at the time of reporting.

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

   Principal Investigator: Michael Antoni, PhD

   Organization: University of Miami

   Progress Report: Research staff have identified a large new sample of potential participants aged 55-59 years old. To consider appropriateness of revising age criterion from ≥ 60 to ≥ 55 years, pilot data were re-analyzed comparing the immune responses to influenza vaccine in different age groups of healthy persons. They compared the hemagglutination inhibition assay (HAI) titer responses of the Hemagglutinin 1 Neurominidase 1' (H1N1) virus to the vaccine among 3 groups: those aged ≤ 40 yrs, those 55-59 years, and those ≥ 60 years. They found that mean vaccine responses (HAI titers) were equivalent between those aged 55- 59 years (M HAI = 344.6 +/- 94.74) and those > 60 years (M HAI = 388.1 +/- 71.14) (p = 0.80), while vaccine

responses in both the 55 – 59 years group ($p < 0.05$) and the > 60 years group ($p < 0.05$) were significantly smaller than the < 40 years group (M HAI = 1120+/- 200.9). These data support that age-related declines in influenza vaccine responses may be present in the period of 55-59 years. Therefore, they determined that revising the age criterion for the present study to ≥ 55 years was scientifically justified. The research team also updated recruitment to identify potential patients based on the study's revised entry criteria (Diagnosis of Breast Cancer in the past 18 months, Stage 0-III, Age ≥ 55 years and English speakers). After meeting with referring physicians, the team implemented recruitment strategies that emphasize working through surgery clinics to initiate patient contact. The participating surgery clinics are in Miami-Dade and Broward counties. Physician recruitment occurred at the Sylvester Comprehensive Cancer Center. A description of the trial has been presented at national meetings and at the Cancer Center's Annual Zubrod Poster and Scientific sessions.

Follow On Funding: None at the time of reporting.

Collaborations: University of Miami, Department of Psychology- two graduate students; School of Medicine- one faculty member; Department of Psychiatry- two faculty members; Department of Microbiology/Immunology- two faculty members.

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: The researchers' central hypothesis is that, in epigenetic silencing, alterations in nucleosome positioning and histone modifications precede hypermethylation of 5'-C-phosphate-G-3' (CpG) islands. To test this hypothesis, the temporal order of changes in chromatin accessibility, histone modification, and CpG methylation will be determined at different stages of *de novo* gene silencing in breast cancer in response to transformation with a dominant, tumorigenic copy of the Harvey rat sarcoma virus (*HRAS*) oncogene. This oncogenic allele of *HRAS* encodes glycine at position 12 instead of the wild-type valine (*HRAS*-V12G), locking the HRAS-V12G enzyme into a pro-growth and pro-tumorigenic state. Clarification of gene-specific pathways of gene repression/silencing will increase understanding about changes in epigenetic landscapes. Moreover, a finding that increased nucleosome occupancy generally precedes deoxyribonucleic acid (DNA) hypermethylation would be impactful, and could lead to using altered nucleosome occupancy as a novel, early biomarker of breast cancer. Aim 1: Determine the global distribution of epigenetic features as a function of breast cancer progression. To address this aim, it was proposed to investigate epigenetic changes across four cell lines that model human breast cancer progression (the "M series" created by Fred Miller at the Karamanos Cancer Institute, Detroit, MI) in Aim 1A-B as well as primary, patient-derived tissues from early-stage breast cancers (Aim 1C). MCF10A cells (hereafter, M1) are non-tumorigenic, immortalized human mammary epithelial cells. M1 cells were transduced with oncogenic *HRAS*, yielding pre-malignant M2 cells (MCF10AT). Introduction of M2 cells into nude mice yielded M3 (MCF10Ca1h) and M4 (MCF10Ca1a) cells, derived from well differentiated, less-aggressive tumor xenografts and poorly differentiated, highly aggressive tumor xenografts, respectively. M series stock was obtained to establish new frozen stocks and

she has performed MAPit. She is currently evaluating the MAPit quality metrics, and when verified, the DNA samples will be shipped to Kappa Biosciences (Boston, MAPit) to perform sequence capture for epigenetic evaluation of >5.5 million CpG sites, “giant” number (SeqCap Epi CpGiant enrichment kit; Roche). Dr. Poudyal will also conduct Assay for Transposase Accessible Chromatin (ATAC-seq) on the M series cells. They will be able to analyze the ATAC-seq results quickly as the data analysis is straightforward; however, they estimate it will take at least three months to mine the SeqCap Epi CpGiant datasets, a typical time-frame for large-scale bisulfite sequencing experiments. ChIP-exo experiments will also be conducted. To validate and analyze chromatin accessibility of target genes/regions identified in Sub-aims 1A-C, they proposed a high-throughput version of MAPit and termed MAPit-patch. At the time of funding, genomic DNA from samples was first digested with one of two commercially available restriction endonuclease that are insensitive to methylation of both CpG and a guanine followed by a cytosine in the 5' → 3' direction of a double-stranded sequence (GpC). This constituted a major constraint on the MAPit-patch assay, in that restriction sites are not typically located where they are most desired, e.g., around transcription start sites and enhancers. A next-generation MAPit-patch assay was developed that avoids the requirement for initial cutting with a restriction enzyme. Currently work is underway to determine the maximum length of amplicons for analysis. To facilitate analysis of MAPit-patch amplicons, researcher has enhanced the unsupervised hierarchical clustering capacities of their bioinformatics pipeline. It was proposed to transfect and transform M1 cells with a conditional doxycycline-inducible copy of a dominant allele of oncogenic HRAS, HRAS-V12G. Conditional expression of the oncoprotein will enable sample gene expression and epigenetic features to capture the stepwise sequence of intermediates that accompany the transition from actively transcribed to epigenetically silenced chromatin. Identifying these silencing intermediates is a strategy found to be very effective in identifying molecular drivers and mechanism of de novo epigenetic silencing. It is well known that doxycycline-inducible transgenes often exhibit leaky expression. Collaboration has occurred to perform high-efficiency genome editing of the endogenous *HRAS* allele from a glycine at position 12 (wild-type) to a valine at position 12, i.e., creating the oncogenic *HRAS*-V12G allele. The team has improved MAPit-patch assay that will be used in this project. Additionally, bioinformatics pipeline is completed that will be used for data analysis.

Follow On Funding: None at the time of reporting.

Collaborations: Kapa Biosystems (Roche); Jonathan Licht, MD, University of Florida

Journals: None at the time of reporting.

Patents: After consultation with their patent attorney, the UF Technology Licensing Office has agreed to file an international application for their improvement to their original MAPit-patch protocol: Methods/kits for targeted cleavage and enrichment of DNA. Mancy H. Nabilsi, PhD and Michael P. Kladde, PhD. Assignee: The University of Florida.

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: The most current aim for this project is to determine androgen-independent, activated cell division control protein 42 homolog (Cdc42)-associated kinase 1 (ACK1)-

dependent recruitment of WD Repeat [a short structural motif of approximately 40 amino acids, often terminating in a tryptophan-aspartic acid (W-D) dipeptide] Domain 5 (WDR5)/ Histone-lysine N-methyltransferase 2D (MLL2) complex to androgen receptor (AR) locus. To ascertain that the recruitment of WDR5/MLL2 methyltransferase complex at the AR locus is dependent on phosphorylation of histone H4 at Tyrosine 88 residue (pY88-H4) epigenetic marks and thus ACK1 kinase activity, C4-2B (it is just a name, there is no long form for it) cells were grown in charcoal stripped media. As control, C4-2B cells were treated with ACK1 inhibitor (R)-9bMS. Subsequently, chromatin extracts were immunoprecipitated with WDR5, MLL2, H3K4me1-3 and ribonucleic acid polymerase II (RNA Pol II) Antibodies, followed by qPCR with AREM1 to 3 and control site primers. To determine the physiological relevance of the pY88-H4 interaction with the WDR5/MLL2 complex, the researchers analyzed the localization of WDR5, MLL2, monomethylated histone H3 lysine 4 (H3K4me1), dimethylated histone H3 lysine 4 (H3K4me2), trimethylated histone H3 lysine 4 (H3K4me3), and RNA Pol II at the AREM1-3 sites. Chromatin immunoprecipitation (ChIP)-quantitative polymerase chain reaction (qPCR) analysis revealed a specific recruitment of WDR5, MLL2, and RNA Pol II, and enrichment of H3K4me3 at the AREM1-3, which was abolished following treatment with ACK1 inhibitor (R)-9bMS (It is a name. R is the one type of the stereoisomer; 9b is the compound number which eventually selected for the studies; MS is the mesylate salt of the 9b compound). In contrast, the deposition of H3K4me1 and H3K4me2 was not impacted by the loss of pY88-H4. Collectively, these data indicate that activated ACK1 establishes a transcriptionally permissive chromatin landscape rich in pY88-H4 and H3K4me3 epigenetic marks to promote AR messenger ribonucleic acid (mRNA) transcription in the androgen-deficient environment of castration-resistant prostate cancers (CRPCs).

Follow On Funding: None at the time of reporting.

Collaborations: Mayo- 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. ACK1 regulates histone H4 Tyr88-phosphorylation and AR gene expression in castration resistant prostate cancer. Cancer Cell. 2017 31(6): 790–803.e8 doi:10.1016/j.ccell.2017.05.003

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: To date, 26 patients have been screened for this study. Two new subjects were enrolled, however one later dropped out. Two subjects were also enrolled at the collaborating center: University of Florida Health Shands Hospital in Jacksonville. Through the screening, two additional patients have been identified as being eligible and potentially interested in participating in the study and will be formally presented with the consent form upon their next scheduled visit. The collection of image data, blood samples, pulmonary function tests, and the Quality of Life surveys have been collected as planned with no deviations to the protocol noted. The Institutional Review Board approved the annual renewal of the protocol.

Follow On Funding: None at the time of reporting.

Collaborations: University of Florida- a PhD student, three masters' students, and pre-med student in Biomedical Engineering; a master's student in Computer Science; and two staff with Medical Physics experience. University of Rochester Medical Center- a clinical Assistant Professor of Radiology

Journals: O'Dell, W., Gormaley, A., Prida, D. Validation of the Gatortail method for accurate sizing of pulmonary vessels from 3D medical images. Med. Phys. 2017 accepted doi:10.1002/mp.12580

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

Bankhead-Coley Cancer Biomedical Research Program

APPENDIX C

FISCAL YEAR 2016-2017 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
5BC02	Mayo Clinic Jacksonville	Radisky, Derek C.	$ 1,200,953	$ 571,784.52	$ 629,168.48	5/24/2015	5/15/2018	No	Yes	No
5BC03	H. Lee Moffitt Cancer Center	Kim, Minjung	$ 970,758	$ 530,511.08	$ 440,246.92	5/19/2015	5/15/2018	No	Yes	No
5BC04	University of Miami	Hu, Jennifer J.	$ 1,290,000	$ 545,261.20	$ 744,738.80	5/25/2015	5/15/2018	No	Yes	No
5BC07	H. Lee Moffitt Cancer Center	Haura, Eric	$ 1,686,887	$ 564,732.31	$ 1,122,154.69	5/25/2015	5/15/2020	No	Yes	Yes
5BC08	Sanford Burnham Medical Research Institute	Perera, Ranjan J.	$ 1,289,948	$ 602,267.13	$ 687,680.87	5/25/2015	5/15/2018	No	Yes	Yes

ACTIVE GRANTS FISCAL YEAR 2016-2017
(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 most effective method to reduce breast cancer mortality is through interventions that can prevent disease development, in combination with early detection of breast cancers which can develop. There have been significant problems, however, with effective implementation of chemoprevention and surveillance strategies. First, while existing endocrine-targeting chemoprevention methods are available and cost-effective, women have been reluctant to use them due to perception of an unfavorable risk/benefit ratio. Additionally, these medications are effective only at reducing estrogen receptor-positive breast cancer. Second, while a routine mammographic screening is very effective at earlier, treatable stages, there is an increased awareness of the cost of mammography and the possibility of overtreatment. The researchers are developing a clinical test that will help focus surveillance and chemoprevention efforts toward women for whom they would be most beneficial. The population that will immediately benefit would be those women who have had a breast biopsy with benign findings. This test, designed by the researchers, utilizes tissue biopsies prepared using common methods for clinical evaluation, making their assay applicable to virtually all women who have had a breast biopsy. They have also developed and optimized experimentally straightforward methods, performable in any clinical laboratory, for isolating molecular information from tissue biopsies. A cost-effective and rapid NanoString-based assay is used to identify molecular markers of risk, which when combined with morphologic and clinical features, results in laboratory staff making accurate, individualized risk predictions. For women at highest risk, the researchers can confidently recommend aggressive surveillance or chemoprevention strategies to keep them safe, while those at minimal risk can be appropriately counseled. This research is also providing a better understanding of how breast cancer develops, which may in the future lead to additional treatments to reduce breast cancer development.

   Follow On Funding: None at the time of reporting.

   Collaborations: University of North Carolina- researcher Dr. Melissa Troester

   Journals: Winham, S.J., Mehner, C., Heinzen, E.P., Broderick, B.T., Stallings-Mann, M., Nassar, A., Vierkant, R.A., Hoskin, T.L., Frank, R.D., Wang, C., Denison, L.A., Vachon, C.M., Frost, M.H., Hartmann, L.C., Thompson, E.A., Sherman, M.E., Visscher, D.W., Degnim, A.C., Radisky, D.C. NanoString-based breast cancer risk prediction for women with sclerosing adenosis. Breast Cancer Res. Treat. 2017 in press doi:10.1007/s10549-017-4441-z

   Stallings-Mann, M.L., Heinzen, E.P., Vierkant, R.A., Winham, S.J., Hoskin, T.L., Denison, L.A., Nassar, A., Hartmann, L.C., Visscher, D.W., Frost, M.H., Sherman, M.E., Degnim, A.C., Radisky, D.C. Postlactational involution biomarkers plasminogen and phospho-STAT3 are linked with active age-related lobular involution. Breast Cancer Res. Treat. 2017 in press doi:10.1007/s10549-017-4413-3

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Visscher, D.W., Frank, R.D., Carter, J.M., Vierkant, R.A., Winham, S.J., Heinzen, E.P., Broderick, B.T., Denison, L.A., Allers, T.M., Johnson, J.L., Frost, M.H., Hartmann, L.C., Degnim, A.C., Radisky, D.C. Breast cancer risk and progressive histology in serial benign biopsies. J. Natl. Cancer Inst. 2017 109(10): tbd doi:10.1093/jnci/djx035

Santen, R.J., Radisky, D.C., Degnim, A., Frost, M.H., Vachon, C.M., Ghosh, K., Guestini, F., McNamara, K.M., Sasano, H. Aromatase expression in atypical ductal hyperplasia in women. Breast Cancer Res. Treat. 2017 163(3): 623-629 doi:10.1007/s10549-017-4184-x. PMID: 28337664

Vierkant, R.A., Degnim, A.D., Radisky, D.C., Visscher, D.W., Heinzen, E.P., Frank, R.D., Winham, S.J., Frost, M.H., Scott, C.G., Jensen, M.R., Ghosh, K., Manduca, A., Brandt, K.R., Whaley, D.H., Hartmann, L.C., Vachon, C.M. Mammographic breast density and risk of breast cancer in women with atypical hyperplasia: an observational cohort study from the Mayo Clinic Benign Breast Disease (BBD) cohort. BMC Cancer. 2017 17(1): 84. doi:10.1186/s12885-017-3082-2 PMID: 28143431. PMCID: PMC5282712.

Degnim, A.C., Hoskin, T.L., Arshad, M., Frost, M.H., Winham, S.J., Brahmbhatt, R., Pena, A., Carter, J.M., Stallings-Mann, M., Murphy, L., Miller, E., Denison, L., Vachon, C.M., Knutson, K.L., Radisky, D.C., Visscher, D.W. Alterations in the immune cell composition in premalignant breast tissue that precede breast cancer development. Clin. Cancer Res. 2017 Jan 26. [Epub ahead of print] doi:10.1158/1078-0432.CCR-16-2026 PMID: 28126725.

Mullooly, M., Ynag, H.P., Falk, R.T., Nyante, S.J., Cora, R., Pfeiffer, R.M., Radisky, D.C., Visscher, D.W., Hartmann, L.C., Carter, J.M., Degnim, A.C., Stanczyk, F.Z., Figueroa, J.D., Garcia-Closas, M., Lissowska, J., Troester, M.A., Hewitt, S.M., Brinton, L.A., Sherman, M.E., Gierach, G.L. Relationship between crown-like structures and sex-steroid hormones in breast adipose tissue and serum among postmenopausal breast cancer patients. Breast Cancer Res. 2017 19(1):8. doi:10.1186/s13058-016-0791-4 PMCID: PMC5244534

Degnim, A.C., Visscher, D.W., Radisky, D.C., Frost, M.H., Vierkant, R.A., Frank, R.D., Winham, S.J., Vachon, C.M., Dupont, W.D., Hartmann, L.C. Breast cancer risk by the extent and type of atypical hyperplasia. Cancer 2016 122: 3087–3088. doi:10.1002/cncr.30151

Degnim, A.C., Dupont, W.D., Radisky, D.C., Vierkant, R.A., Frank, R.D., Frost, M.H., Winham, S.J., Sanders, M.E., Smith, J.R., Page, D.L., Hoskin, T.L., Vachon, C.M., Ghosh, K., Hieken, T.J., Denison, L.A., Carter, J.M., Hartmann, L.C., Visscher, D.W. Extent of atypical hyperplasia stratifies breast cancer risk in 2 independent cohorts of women. Cancer 2016 122: 2971–2978. doi:10.1002/cncr.30153

Radisky, D.C., Visscher, D.W., Frank, R.D., Vierkant, R.A., Winham, S., Stallings-Mann, M., Hoskin, T.L., Nassar, A., Vachon, C.M., Denison, L.A., Hartmann, L.C., Frost, M.H., Degnim,

A.C. Natural history of age-related lobular involution and impact on breast cancer risk. Breast Cancer Res.Treat. 2016 155(3): 423–430. doi:10.1007/s10549-016-3691-5

Patents: None at the time of reporting.

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

   Principal Investigator: Minjung Kim, PhD

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: This project seeks to understand the role of a small Guanosine-5'-triphosphate (GTP)-binding protein, influences integrin activation (R-Ras) in melanoma tumorigenesis. The research team has addressed molecular mechanisms on how a serine/threonine protein kinase activating the MAP kinase/ERK-signaling pathway (BRAF) activation cooperates with R-Ras signaling. Staff tested whether R-Ras is phosphorylated on serine (Ser) in BRAF mutant melanoma cells using a phosphor-PXpS/TP motif [consensus sequence of Mitogen-Activated Protein Kinase (MAPK) substrate] antibody. They have observed that R-Ras is phosphorylated on Ser in WM983C (BRAF mutant) human melanoma cells and inhibition of MAPK via vemurafenib (BRAF inhibitor) or trametinib (MEK inhibitor) suppressed R-Ras phosphorylation of Ser on MAPK consensus sequence, which correlated with decreased R-Ras. This suggests that R-Ras activity is regulated by MAPK as well as RasGAPs and provides possible explanation for the cooperative interaction of BRAF activation and RASA1 (RasGAP) inactivation in melanoma. They will generate R-Ras S201A and S201D mutants to directly address this hypothesis (role of BRAF activation on R-Ras activation). To address tumor maintenance role of R-Ras, they have generated tet-inducible shRNAs targeting R-Ras in the lentiviral vector, pLKO-tet-R, plasmids (Aim 3). In Aim 4, they continued monitoring of the RASA1 tumor cohort for melanoma formation. Among the mice enrolled nine months or longer, they observed cutaneous melanomas in 4/40 BR (Rasa1 heterozygous mutant mice (one copy loss with BRAF activation) and 4/33 BRR (Rasa1 homozygous mutant mice (both copy loss with BRAF activation) mice, but 0/14 in B (Rasa1 wild-type mice with BRAF activation) mice. They observed deaths of several mice in each group without any obvious tumors. Histological analyses found few cases of diffuse idiopathic pulmonary neuroendocrine cell hyperplasia in lung. Interestingly, even in mice without macroscopic melanomas, they observed several small foci of melanoma cells 100% soluble in ammonium sulfate at neutral pH (S100) positivity in skin. Therefore, skin of all the mice will be thoroughly analyzed for these microscopic tumors by haematoxylin eosin and S100 immunohistochemistry.

   Follow On Funding: None at the time of reporting.

   Collaborations: Florida Gulf Coast University- a research associate and a recent graduate; University of South Florida- a research intern and a recent graduate

   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. 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.

3. **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: The laboratory staff proposed to use the newly developed Infinium OncoArray-500K BeadChip (contains 500,000 single-nucleotide polymorphisms). They have recruited new patients; genomic deoxyribonucleic acid has been isolated from whole blood ready for genotyping. A batch of genotyping assay in 1,211 samples is completed and performed a quantity check. During quantitation process, they identified some samples with lower concentration than the required concentration for the OncoArray protocol, so they have been working on optimizing concentration before submitting the samples to the cores for genotyping. The first batch of genotyping data collection (n=576) is being completed. With additional patient recruitment, they plan to finish the second batch of 672 samples in February 2018. Frozen plasma samples will be used for metabolomics assay. Collaborators at the University of Florida Southeast Resource Center for Integrated Metabolomics have completed the global metabolomics assay development, validation, and automation process to be ready for large population and clinical research projects. They will continue to use the non-targeted metabolic profiling instrumentation employed for this analysis with three combined independent platforms: ultrahigh performance liquid chromatography/tandem mass spectrometry optimized for basic species and acidic species, as well as gas chromatography/mass spectrometry. To minimize batch-to-batch variation, the plasma samples will be delivered batches of 48 samples (i.e., 18 controls, 10 estrogen receptor+ cases, 10 estrogen receptor-/human epidermal growth factor receptor 2+ cases, and 10 triple negative breast cancer). With their newly installed automatic extraction and sampling system in place, they anticipate that all metabolomics assays (six batches) will be completed. Illumina TruSight Ribonucleic Acid Pan-Cancer panel will be used, which provides a comprehensive analysis of the cancer transcriptome. Targeting 1,385 cancer-related transcripts and genes known to be involved in gene fusions, this approach enables analysis of cancer samples including archived formalin-fixed, paraffin-embedded tissues and other limited samples. This targeted panel offers: (i) gene expression information, variant calling, and fusion detection with known and novel gene fusion partners; (ii) optimized, low input protocol for a wide range of sample types including formalin-fixed, paraffin-embedded; (iii) a comprehensive view of cancer pathways; and (iv) economical ribonucleic acid sequencing on a desktop sequencer. To date, they have completed ribonucleic acid isolation and quality/quantity check of the first 96 snap frozen biopsies, the Cancer Center Oncogenomics core facility has started working on the TruSight® Ribonucleic Acid Pan-Cancer panel next-generation sequencing assay; the software provided by Illumina will be extremely efficient in generating the final somatic mutation data. After validating somatic mutation data in 10 paired formalin-fixed, paraffin-embedded samples, the plan is to retrieve formalin-fixed, paraffin-embedded samples using automatic extraction for the remaining assay to reach the sample size of 480. The next-generation sequencing assays (5 batches) will be completed in February 2018. Overall significant progress has been made, particularly inpatient enrollment (total N=1,374; 764 controls and 610 breast cancer cases) and biopsy sample collection (total N=459).

Follow On Funding: None at the time of reporting.

Collaborations: University of Florida Southeast Resource Center for Integrated Metabolomics (SECIM)- three MSPH students and a PhD student.

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. A functionally significant SNP in TP53 and breast cancer risk in African American women. NPJ Breast Cancer. 2017 3:5. doi:10.1038/s41523-017-0007-9. eCollection 2017. PubMed PMID: 28649645; PubMed Central PMCID: PMC5445618

Nagel, Z.D., Engelward, B.P., Brenner, D.J., Begley, T.J., Sobol, R.W., Bielas, J.H., Stambrook, P.J., Wei, Q., Hu, J.J., Terry, M.B., Dilworth, C., McAllister, K.A., Reinlib, L., Worth, L., Shaughnessy, D.T. Towards precision prevention: Technologies for identifying healthy individuals with high risk of disease. Mutat. Res. 2017 800-802:14-28. doi:10.1016/j.mrfmmm.2017.03.007. [Epub ahead of print] PubMed PMID: 28458064

Feng, Y., Rhie, S.K., Huo, D., Ruiz-Narvaez, E.A., Haddad, S.A., Ambrosone, C.B., John, E.M., Bernstein, L., Zheng, W., Hu, J.J., Ziegler, R.G., Nyante, S., Bandera, E.V., Ingles, S.A., Press, M.F., Deming, S.L., Rodriguez-Gil, J.L., Zheng, Y., Yao, S., Han, Y.J., Ogundiran, T.O., Rebbeck, T.R., Adebamowo, C., Ojengbede, O., Falusi, A.G., Hennis, A., Nemesure, B., Ambs, S., Blot, W., Cai, Q., Signorello, L., Nathanson, K.L., Lunetta, K.L., Sucheston-Campbell, L.E., Bensen, J.T., Chanock, S.J., Marchand, L.L., Olshan, A.F., Kolonel, L.N., Conti, D.V., Coetzee, G.A., Stram, D.O., Olopade, O.I., Palmer, J.R., Haiman, C.A. Characterizing genetic susceptibility to breast cancer in women of African ancestry. Cancer Epidemiol. Biomarkers Prev. 2017 26(7):1016-1026. doi:10.1158/1055-9965.EPI-16-0567. Epub 2017 Apr 4. PubMed PMID: 28377418

Wright, J.L., Takita, C., Reis, I.M. Zhao, W., Lee, E., Nelson, O.L., Hu, J.J. Prospective evaluation of radiation-induced skin toxicity in a race/ethnically diverse breast cancer population. Cancer Med. 2016 5(3): 454-464 doi:10.1002/cam4.608. PubMed PMID: 26763411

Lee, E., Takita, C., Wright, J.L., Reis, I.M., Zhao, W., Nelson, O.L., Hu, J.J. Characterization of risk factors for adjuvant radiotherapy-associated pain in a tri-racial/ethnic breast cancer population. Pain 2016 157(5):1122-31. doi:10.1097/j.pain.0000000000000489 PubMed PMID: 26780493

Rand, K.A., Song, C., Dean, E., Serie, D.J., Curtin, K., Sheng, X., Hu, D., Huff, C.A. Bernal-Mizrachi, L., Tomasson, M.H., Ailwadhi, S., Singhai, S., Pawlish, K.S., Peters, E.S., Block, C.H., Stram, A., Van Den Berg, D.J., Edlund, C.K., Conti, D.V., Zimmerman, T.M., Hwang, A.E., Huntsman, S., Graff, J.J., Nooka, A., Kong, Y., Pregja, S.L., Berndt, S.I., Blot, W.J., Carpten, J.D., Casey, G., Chu, L.W., Diver, W.R., Stevens, V.L., Lieber, M.R., Goodman, P.J., Hennis, A.J., Hsing, A.W., Mehta, J., Kittles, R.A., Kolb, S., Klein, E.A., Leske, C.M., Murphy, A.B., Nemesure, B., Neslund-Dudas, C., Strom, S.S., Vij, R., Rybicki, B.A., Stanford, J.L., Signorello, L., Witte, J.S., Ambrosone, C.B., Bhatti, P., John, E.M., Bernstein, L., Zheng, W., Olshan, A.F., Hu, J.J., Ziegler, R.G., Nyante, S.J., Bandera, E.V., Birmann, B.M., Ingles, S.A., Press, M.F., Atanackovic, D., Glenn, M., Cannon-Albright, L., Jones, B., Tricot, G., Martin, T.G., Kumar, S.K., Wolf, J.L., Deming, S.L., Rothman, N., Brooks,-Wilson, A., Rajkumar, S.V., Kolonel, L.N., Chanock, S.J., Slager, S.L., Severson, R.K., Janakirman, N., Terebelo, H.J., Brown, E.E., De

Roos, A.J., Mohrbacher, A., Colditz, G.A., Giles, G.G., Spinelli, J.J., Chiu, B.C., Munshi, N.C., Anderson, K.C., Levy, J., Zonder, J.A., Orlowski, R.Z., Lonial, S., Camp, N.J., Vachon, C.M., Ziv, E., Stram, D.O., Hazelett, D.J., Cozen, W. A meta-analysis of multiple myeloma risk regions in African and European ancestry populations identifies putatively functional loci. Cancer Epidemiol. Biomarkers Prevention 2016. 25(12):1609-1618 pii:cebp 1193.2015 doi:10.1158/1055-9965.EPI-15-1193 PubMed PMID: 27587788

Huo, D., Feng, Y., Haddad, S., Zheng, Y., Yao, S., Han, Y.J., Ogundiran, T.O., Adebamowo, C., Ojengbede, O., Falusi, A.G., Zheng, W., Blot, W., Cai, Q., Signorello, L., John, E.M., Bernstein, L., Hu, J.J., Ziegler, R.G., Nyante, S., Bandera, E.V., Ingles, S.A., Press, M.F., Deming, S.L., Rodriguez-Gil, J.L., Nathanson, K.L., Domcheck, S.M., Rebbeck, T.R., Ruiz-Narváez, E.A., Sucheston-Campbell, L.E., Bensen, J.T., Simon, M.S., Hennis, A., Nemesure, B., Leske, M.C., Ambs, S., Chen, L.S., Qian, F., Gamazon, E.R., Lunetta, K.L., Cox, N.J., Chanock, S.J., Kolonel, L.N., Olshan, A.F., Ambrosone, C.B., Olopade, O.I., Palmer, J.R., Haiman, C.A. Genome-wide association studies in women of African ancestry identified 3q26.21 as a novel susceptibility locus for estrogen receptor negative breast cancer. Hum. Mol. Genet. 2016. 25(21): 4835-4846 doi:10.1093/hmg/ddw305 pii:ddw305. PubMed PMID: 27594435

Qian, F., Feng, Y., Zheng, Y., Ogundiran, T.O., Ojengbede, O., Zheng, W., Blot, W., Ambrosone, C.B., John, E.M., Bernstein, L., Hu, J.J., Ziegler, R.G., Nyante, S., Bandera, E.V., Ingles, S.A., Press, M.F., Nathanson, K.L., Hennis, A., Nemesure, B., Ambs, S., Kolonel, L.N., Olopade, O.I., Haiman, C.A., Huo, D. Genetic variants in microRNA and microRNA biogenesis pathway genes and breast cancer risk among women of African ancestry. Hum. Mol. Genet., 2016. 135(10): 1145-59. doi:10.1007/s00439-016-1707-1. Epub 2016 Jul 5. PubMed PMID: 27380242

Ruiz-Narváez, E.A., Sucheston-Campbell, L., Bensen, J.T., Yao, S., Haddad, S., Haiman, C.A., Bandera, E.V., John, E.M., Bernstein, L., Hu, J.J., Ziegler, R.G., Deming, S.L., Olshan, A.F., Ambrosone, C.B., Palmer, J.R., Lunetta, K.L. Admixture mapping of African-American women in the AMBER consortium identifies new loci for breast cancer and estrogen-receptor subtypes, Front Genet. 2016. 7:170. doi:10.3389/fgene.2016.00170 PubMed PMID: 27708667

Patents: None at the time of reporting.

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

   Principal Investigator: Eric Haura, MD

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: Cell lines have been established that are resistant to the third-generation epidermal growth factor receptor (EGFR) inhibitor, osimertinib (AZD9291). The Moffitt research team started from two different parental cell lines: PC9 which are sensitive to erlotinib and PC9GR, which is resistant to erlotinib due to the presence of the T790M “gatekeeper” mutation. Initially, both lines were sensitive to osimertinib, but the researchers have now generated resistant clones that are growing in 1 micromolar osimertinib. It was hypothesized that different modalities of resistance could emerge depending on whether or not the gatekeeper mutation is

present. This approach mimics resistance mechanisms that may emerge in the ongoing phase III trial that is comparing erlotinib to osimertinib in the first line setting (NCT02296125). The researchers have found that the resistant phenotypes are stable in the absence of drug up to four weeks. They have generated single cell clones (N =12) for each resistant population and are attempting to determine if multiple resistance is mediated by different mechanisms in these clones. Using the polyclonal populations, the researchers have two preliminary findings that are currently being followed. It was observed that the resistant lines lose dependence on the growth factor receptor-bound protein 2 (GRB2) adaptor protein, but maintain a dependency on the Src Homology 2 Domain-Containing Transforming Protein 1 (SHC1) adaptor protein. This suggests that a SHC1-containing protein complex may be critical for mediating the resistant phenotype. It was also observed that resistant cells acquire sensitivity to kinase inhibitors crizotinib and TAE684, but not PHA665752. This suggests that the receptor tyrosine kinase encoding the hepatocyte growth factor receptor (cMET) is not mediating the resistance. Crizotinib and a 2,4-dianilino-5-chloro-pyrimidine (TAE684) are known to target anaplastic lymphoma kinase (ALK), but these cell lines do not express ALK. Thus, they are likely observing “off target” effects of these inhibitors and are currently investigating additional candidates such as focal adhesion kinase (FAK) and tyrosine-family kinases. The researchers plan to further characterize these cell line models of acquired resistance and to determine if novel signaling networks and signaling-associated complexes are involved in the maintenance of the resistant phenotypes. Using proximity ligation assay-based approaches will enable them to assess if targetable kinases are mediating resistance. It is also intended to apply these assays in patient specimens that become resistant to osimertinib. A large cohort of patients treated with EGFR tyrosine kinase inhibitors is being assembled to facilitate studies in human biopsies. Because reversion to wildtype EGFR signaling is a known resistance mechanism, the research team hypothesize that assays for EGFR signaling complexes will have utility in this setting because it could inform the use of a first-generation EGFR inhibitor such as erlotinib in combination with osimertinib for some patients.

Follow On Funding: National Cancer Institute - $390,763

Collaborations: Albert Einstein Medical School; Weill-Cornell Medical School; University of Colorado; and Oncotest in Germany. A collaborative agreement is in effect with the Tissue Research & Early Development (tRED) division of Ventana.

Journals: Smith, M.A., Licata, T., Likhani, A., Garcia, M.V., Schildhaus, H-U., Vuaroqueaux, V., Halmos, B., Borczuk**,** A.C., Creelan, B.C., Boyle, T., Haura, E.B. MET-GRB2 signaling-associated complexes correlate with oncogenic MET signaling and sensitivity to MET kinase inhibitors. Clinical Can. Res. August 29, 2017 doi:10.1158/1078-0432.CCR-16-3006

Vaishnavi, A., Schubert, L., Rix, U., Marek, L.A., Le, A.T., Keysar, S.B., Glogowska, M.J., Smith, M.A., Kako, S., Sumi, N.J. Davies, K.D., Ware, K.E., Varella-Garcia, M., Haura, E.B., Jimeno, A., Heasley, L.E., Aisner, D.L. and Doebele, R.C. EGFR mediates responses to small-molecule drugs targeting oncogenic fusion kinases. Cancer Res. 77(13) 3551–3563 doi:10.1158/0008-5472.CAN-17-0109

Patents: None at the time of reporting.

5. **Grant #5BC08:** The Expansion and Update 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: Research staff purchased a Pipin Prep DNA size selection machine to support experimental flow. This platform facilitates efficient library construction for most popular Next-Generation Sequencing (NGS) platforms, and was recommended by Illumina Corp. Target sizes or ranges of sizes are entered in software, and fractions are collected in buffer. Several new collaborators from Florida International University (FIU), University of Central Florida (UCF) and Sanford-Burnham Prebys. Medical Discovery Institute (SBP) would like to see that SBP genomics core develop single cell sequencing capabilities. Post-doctoral fellow and core members have already begun to evaluate Fludigm single cell isolation and library preparation machine. Cancer genomics medicine symposium was held March 2017 and a major success. A follow-up symposium was recommended for 2018. Key opinion leaders from the University of Florida (UF), H. Lee Moffitt Cancer Center, the University of Miami, FIU, UCF, the University of South Florida and John Hopkins All Children's Hospital unanimously agreed to organize the next symposium. They hope to organize the next symposium at UF. Prior to the symposium, another mini-symposium was held for Florida genomics core Directors.

Follow On Funding: National Institute of Health - $466,538 and Florida Breast Cancer Foundation - $199,937

Collaborations: Bankhead Coly funded post-doc (Dr. Lee); Florida International University (Dr. Yuk-Ching Tse-Dinh); University of Central Florida (Dr. Ratna Chakrabarti); Florida International University (Dr. Fenfei Leng); University of Florida (Dr. Daiqing Liao and Dr. Daaka); Sanford Burnham Prebys (Dr. Masanobu Komatsu and Dr. Laszlo Nagy)

Journals: Sahoo, A., Boniface, K., Seneschal, J., Sahoo, S.K., Seki, T., Wang, C., Bas, S., Han, X., Steppie, M., Seal, S., Taieb, A., Perera, R.J., MicroRNA-211 regulates oxidative phosphorylation and energy metabolism in human vitiligo. J. Invest. Dermatol. 2017. 137(9): 1965-1974 doi:10.1016/j.jid.2017.04.025

Bongyong L., Sahoo, A., Marchica, J., Holzhauser, E., Chen, X., Li, J-L., Seki, T., Govindarajan, S.S., Markey, F.B., Batish, M., Lokhande, S.J., Zhang, S., Ray, A., and Perera, R.J. The long noncoding RNA SPRIGHTLY acts as an intranuclear organizing hub for pre-mRNA molecules. Science Advances. May 2017: 3(5), e1602505 doi: 10.1126/sciadv.1602505

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Zhao, W., Mazar, J., Lee, L., Sawada, J., Li, J-L., Shelley, J., Govindarajan, S., Towler, D., Mattick, J.S., Komatsu, M., Dinger, M.E., and Perera, R.J. The long noncoding RNA SPRIGHTLY regulates cell proliferation in primary human melanocytes. Jour. Invest. Dermatol. 2016. 136(4): 819-828, doi:10.1016/j.jid.2016.01.018

Patents: None at the time of reporting.

Bankhead-Coley Cancer Biomedical Research Program

APPENDIX D

FISCAL YEAR 2016-2017 COMPLETED GRANTS,
Funding Fiscal Year 2015-2016

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
6BC01	University of Florida	Ishov, Alexander M.	$ 100,000	$ 99,438.67	$ 561.33	3/18/2016	8/31/2016	No	No	Yes
6BC03	University of Florida	Liao, Daiqing	$ 100,000	$ 99,250.87	$ 749.13	3/08/2016	2/28/2017	Yes	Yes	No

COMPLETED GRANTS FISCAL YEAR 2016-2017

(Funding Year 2015-2016)

1. **Grant #6BC01:** Functions of Histone Variants in Castration-Resistance Prostate Cancer

   Principal Investigator: Alexander M. Ishov, PhD

   Organization: University of Florida

   Progress Report: Androgen receptor (AR) mediates initiation/progression of prostate cancer (PCa); it is activated by testosterone/dihydrotestosterone that promotes AR nuclear relocation, binding to AR response elements (ARE) and activation of AR-dependent genes. Androgen ablation therapies offer a temporary relief and the disease eventually recurs as castration-resistant PCa (CRPC). A better understanding of mechanisms that control AR activation and allow CRPC to circumvent hormonal ablation therapies is critically important for improving disease outcome. To further address the question whether death-associated protein 6 and another non-centromeric histone H3 variants (H3.3) chaperone histone cell cycle regulator (HIRA) deposit H3.3 and regulate C-terminally truncated androgen receptor (AR) variants lacking vast parts of the ligand-binding domain (ARΔLBD) expression, gene editing in human cells was performed using state-of-the-art Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas9) methodology. With this approach, R1-J and R1-446 cells (AR-Wild Type and ARΔLBD) were engineered that lack the cell death receptor-binding protein (Daxx) or HIRA. After first screening clones by microscopy analysis, six clones of Daxx-/- cells and one for HIRA-/- cells, were expanded and cryopreserved. Confirmation of the disruption of the open reading frames by sequence analysis and Western blot analysis confirmed absence of Daxx or HIRA expression. These cells will be used for further functional studies. The ratio between H3.1 and H3.3 endogenous expression can have dramatic effects on histone variant deposition and chaperone-variant specificity. CRISPR/Cas9-based knock in to generate prostate cancer cells with a polypeptide protein human influenza hemagglutinin-tagged (FLAG/HA-tagged) allele of encoding H3.3 gene. FLAG/HA-H3.3 expression in R1-J and R1-446 cells was analyzed by immunofluorescence (IFA). Several single-cell colonies were isolated and characterized by IFA and Western Blot (immunoblotting or protein blotting method used to detect specific proteins). These cell lines allowed the investigators to directly determine whether Daxx and/or HIRA affects endogenous H3.3 deposition onto ARE. Interaction between ARΔLBD and Daxx recruits histone chaperone activity to ARE, changing AR-driven expression profile that forces CRPS progression. Experiments were started to map Daxx/ARΔLBD interaction to identify interference strategy for CRPS. In summary, good progress was made creating several reagents that will be suggested for use in future submission of a Research Project Grant by the National Institute of Health/National Cancer Institute.

   Follow On Funding: National Cancer Institute - $358,875

   Collaborations: None at the time of reporting.

   Journals: None at the time of reporting.

   Patents: None at the time of reporting.

2. **Grant #6BC03:** Target HDAC2 for Treating ER-Positive and Drug-Resistant Breast Cancer

   Principal Investigator: Daiqing Liao, PhD

Organization: University of Florida

Progress Report: This pilot experiment was conducted to identify suitable antibodies against HDAC2 and ER for immunoprecipitation. During the grant period, the laboratory staff have successfully generated histone deacetylase 2 (HDAC2) knockout in several estrogen receptor + breast cancer (ER+BC) cell lines, and have assessed whether the absence of HDAC2 expression would sensitize ER+BC cell to endocrine therapies. Suitable antibodies have been identified and the assessment of HDAC2-ER interaction using immunoprecipitation was conducted. Several BC cell lines were obtained from the American Type Culture Collection and used to expand the cancer cell culture. Experiments were conducted to determine the effects of the HDAC inhibitors on cell viability and whether these inhibitors can enhance cancer cell death in combination with endocrine therapies. Further testing was proposed to determine if the new inhibitors could suppress tumor growth *in vivo* using a patient derived xenograft tumor model.

Follow On Funding: None at the time of reporting.

Collaborations: Two PhD students and several undergraduate students at the University of Florida performed research under this project.

Journals: Wang, Y., Li, D., Luo, J., Tian, G., Zhao, L.Y. and Liao, D. Intrinsic cellular signaling mechanisms determine the sensitivity of cancer cells to virus-induced apoptosis. 2016. Sci. Rep 6: 37213, doi:10.1038/srep37213

Liao, D. CBP/p300 Bromodomain mediates amyloid formation. Cell Chem. Biol. 2017. 24:128-129, doi:10.1016/j.chembiol.2017.01.004

Patents: Patent application (PCT/US2015/023437): HDAC Inhibitor Compounds and Methods of Treatment.

Bankhead-Coley Cancer Biomedical Research Program

APPENDIX E

FISCAL YEAR 2016-2017 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
5BC01	H. Lee Moffitt Cancer Center	Lynch, Conor	$ 1,290,000	$ 219,722.38	$ 1,070,277.62	5/25/2015	6/08/2016	No	Yes	Yes
5BC05	University of Florida	Wu, Lizi	$ 86,000	$ 76,903.09	$ 9,096.91	5/25/2015	11/15/2015	No	No	No

COMPLETED GRANTS FISCAL YEAR 2016-2017
(Funding Year 2014-2015)

1. **Grant #5BC01:** An Integrated Computational and Biological Approach to Curing Prostate to Bone Metastases

   Principal Investigator: Conor Lynch, PhD

   Organization: H. Lee Moffitt Cancer Center and Research Institute

   Progress Report: The focus of this grant was to test the role of transforming growth factor beta (TGFb) in the context of bone metastatic prostate cancer to better understand the pathways involved in cancer-bone communication. Specific Aim 1: Understanding how prostate cancer cells utilize TGFb. The research team focused on homogenous prostate cancer cells expressing and producing both the receptor and the ligand. Using an existing computational model, they tested the impact of TGFb inhibition on the prostate cancer cells. The results from the model indicated that pre-treatment of these lesions with TGFb inhibition impacts the growth of the disease *in silico* and *in vivo* both directly and indirectly. Examining the effects of TGFb inhibition on different clonal populations, they reprogrammed the *in silico* model and used the C4-2B cell line to address the effect of TGFb inhibition on cancer cells that produce the TGFb ligand but does not express the receptor. Based on the results from the human specimens, there is a wide variation in how metastatic prostate cancer cells utilize TGFb. Cancer cells that express both the receptor and produce the ligand (TRP), produce only the receptor (TR), only the ligand (TP), or neither (TN) has an average ratio across several specimens of 231:6:1:4 respectively. Since the dominant population in human bone metastases is the TRP population, this indicates that the application of TGFb inhibitors, would be beneficial for the treatment of the disease. This information resulted in the development of new partial differential equations that dictate the behavior of each of those clones and integrate them at the above ratio into their *in silico* model. Under control conditions they have found that TRP cancer cells are the dominant population of clones at the end of simulations. Using this information, staff applied the TGFb inhibitor in an adaptive manner to prevent the emergence of the TN population and extend the time line of the model. They planned to apply an osteoclast inhibitor (anti-RANKL) at pre-influx time points to determine if it can mitigate cancer induced bone destruction (aim 2) and examine the importance of mesenchymal stromal cells (MSCs) in driving prostate cancer induced osteogenesis (aim3). However, before the research team could begin aims 2 and 3, they were awarded a grant from the National Institutes of Health that conflicted with their Florida Department of Health (FDOH) Bankhead-Coley research grant, and subsequently relinquished their FDOH award in June 2016.

   Follow On Funding: National Institute of Health - $3,200,000

   Collaborations: None at the time of reporting.

   Journals: Cook LM, Araujo A, Pow-Sang JM, Budzevich MM, Basanta D, Lynch CC. Predictive computational modeling to define effective treatment strategies for bone metastatic prostate cancer. Sci Rep. 2016 Jul;6:29384. doi:10.1038/srep29384 Pubmedid: 27411810. Pmcid: PMC4944130.

   Patents: None at the time of reporting.

2. **Grant #5BC05:** Molecular Regulation of CNS Leukemia Development

   Principal Investigator: Lizi Wu, PhD

   Organization: University of Florida

   Progress Report: With the goal of gaining molecular insights into leukemia pathogenesis and identifying novel cancer diagnostic and therapeutic targets, the research team performed expression and functional studies of genes associated with brain-derived leukemia (aim1). To accomplish this specific task, the team developed and used a second mouse model with bioluminescence imaging (BLI)-detectable central nervous system (CNS) leukemia, and expressed the leukemia cell line cells with luciferase (CEM-luc) after pre-treatment. After which, the team analyzed the cells upon isolating them from the brain, spinal cord, and bone marrow for cell proliferation rates and gene expression. Based on results, researchers determined that lin-28 homolog B protein (LIN28B) does up-regulate leukemia cell growth and enhances the overexpression of cell proliferation and migration. Gene expression profiling analysis was performed to identify LIN28B target gene pathways. The second portion of the bridge funding was used to evaluate the angiogenic factors VEGFA and ANGPT2 (or Vascular Endothelial Growth Factor A and Angiopoietin-2, respectively) in CNS leukemia (aim 2). This was devised to determine whether anti-VEGFA therapeutics block the development of CNS leukemia. Results have been validated of VEGFA and ANGPT2 knockdown *in vivo*, and prepared to test the luciferase-expressing human thymic acute lymphoblastic leukemia (HPB-ALL-luc) cell line *in vivo*. Due to a delay in recruiting a post-doctorate researcher to complete the *in vivo* experiment, they were unable to complete the proposed drug treatment testing related to aim 2.

   Follow On Funding: None at the time of reporting.

   Collaborations: None at the time of reporting.

   Journals: None at the time of reporting.

   Patents: None at the time of reporting.

James & Esther King Biomedical Research Program

APPENDIX F

FISCAL YEAR 2016-2017 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
7JK01	University of Miami	Bramlett, Helen M.	$ 1,253,753	$ 61,735.07	$ 1,192,017.93	3/09/2017	2/29/2020	No	No	No
7JK02	H. Lee Moffitt Cancer Center	Chung, Christine	$ 1,896,200	$ 59,361.37	$ 1,836,838.63	3/16/2017	2/28/2022	No	No	No
7JK03	University of Miami	Dietrich, W. Dalton	$ 941,589	$ 44,987.27	$ 896,601.73	3/08/2017	2/29/2020	No	No	No
7JK04	H. Lee Moffitt Cancer Center	Gray, Jhanelle	$ 1,895,355	$ 14,104.20	$ 1,881,250.80	3/25/2017	2/28/2022	No	No	No
7JK05	University of Florida	Jiang, Zhihua	$ 1,422,150	$ 2,852.93	$ 1,419,297.07	3/07/2017	2/29/2020	No	No	No
7JK06	Florida A&M University	Sachdeva, Mandip S.	$ 94,810	0	$ 94,810	3/27/2017	3/31/2018	No	No	No
7JK07	University of Florida	Fan, Z. Hugh	$ 125,000	0	$ 125,000	6/15/2017	6/30/2018	No	No	No

NEWLY AWARDED GRANTS FISCAL YEAR 2016-2017

(Funding Year 2016-2017)

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

   Principal Investigator: Helen M. Bramlett, PhD

   Organization: University of Miami

   Abstract of Proposed Research: Millions of smokers are disabled as a result of stroke and ischemic stroke accounts for almost 85% of total stroke cases. Ischemic stroke occurs when the blood supply to part of the brain is disrupted due to thromboembolic occlusion of a cerebral artery. Disruption of blood supply to part of the brain causes focal ischemia damaging the cortical region initially. To date, the only drug that has been approved to treat acute stroke is the clot-dissolving drug tissue plasminogen activator (tPA). However, tPA must be administered within 3 hours of the onset of an ischemic stroke, which makes it a viable treatment for less than 15% of stroke patients. Thus, new therapies for acute stroke with extended therapeutic windows are badly needed. Physical therapy and exercise have been shown to be beneficial for recovery but often are not an option for frail patients. Whole Body Vibration (WBV) mimics the internal forces exerted on by exercise, and can be effectively incorporated in any patient's treatment regimen. Although WBV has been previously shown to be beneficial in maintenance and increase of bone mass, in this study the research team wants to test its direct application in the recovery from stroke. They hypothesize that WBV will significantly improve cognition, inflammation and neuron growth in nicotine exposed rats after stroke.

   Follow On Funding: None at the time of reporting.

   Collaborations: Mr. Nathan d'Adesky is a first-year medical student at the University of Florida. He is a summer research fellow in their laboratory. He is receiving training and currently performing research under the project.

   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 Chung, MD

   Organization: H. Lee Moffitt Cancer Center

   Abstract of Proposed Research: Head and neck squamous cell carcinoma (HNSCC) remains one of the most devastating cancers affecting oral cavity, oropharynx, hypopharynx, and larynx that are critical structures for life's most essential functions such as eating, breathing, and talking. Common risk factors are tobacco and alcohol use and human papillomavirus (HPV) infection. The patients with tobacco-related HNSCC have the worst prognosis compared to those with HPV-related HNSCC. Recently immunotherapy has become a promising therapeutic option in HNSCC. Among the numerous immunotherapeutic agents, programmed cell death-1 (PD-1) inhibitors are the most advanced in development in HNSCC, particularly pembrolizumab and nivolumab. PD-1 is an important protein that regulates the immune cell functions which are

critical in recognizing and eliminating the abnormal cancer cells. Activation of PD-1 can decrease this immune function by suppressing T cells. Thus, inhibiting PD-1 improves the ability of T cells to fight the cancer. These immunotherapy agents set themselves apart from chemo- and other therapies by their ability to induce long lasting clinical benefits leveraging the patient's own immune system; however, the efficacy is seen only in a limited number of patients. It is imperative to identify patients who will truly benefit from these immunotherapy agents, to improve the current response to immunotherapy, and accurately assess the toxicities moving towards more personalized therapies. In this project, the study team proposes; 1) to identify predictive biomarkers to select the patients who will benefit the most from current PD-1 inhibitors based on their tumor genetic alterations that may trigger the immune response, 2) to determine whether combining cetuximab, a currently FDA-approved cancer drug, and nivolumab will improve the immune response and clinical benefits, 3) to determine changes in the tumor infiltrating immune cells induced by tobacco use, 4) to determine changes in the tumor genes and proteins induced by tobacco use in order to find new drug targets, and 5) to develop a smartphone-based assessment for real-time reporting of toxicities and tobacco use by the patients between the clinic visits. The findings from this project will have a significant impact on the health of Floridians by reducing mortalities through improving the selection of patients who will gain the most benefit from receiving PD-1 inhibitors, reducing mortalities by a novel combination therapy, and reducing morbidities of treatment-related toxicities and on-treatment tobacco use through development of a real-time assessment by patient reporting.

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 #7JK03:** The Therapeutic Effect of P7C3-A20 on Stroke

   Principal Investigator: W. Dalton Dietrich, PhD

   Organization: University of Miami

   Abstract of Proposed Research: Focal cerebral ischemia leading to stroke is a devastating condition that has few therapeutic interventions available except for early thrombolytic therapy or new catheter-based endovascular strategies. There is a major need to develop and test new pharmacological agents to protect neurons from irreversible cell death. In addition to cell death, several studies have reported an increase in the generation of new neurons in specific brain regions following focal cerebral ischemia. Therapeutic strategies that also protect these newly formed neurons from death, would potentially promote functional recovery after stroke. The recently identified proneurogenic compound P7C3-A20 has been reported to inhibit neuronal cell death, enhance the formation of new neurons and improve cognitive function in several neurodegenerative models. The goal of this project is to determine whether treatment with P7C3-A20 at various periods after the focal ischemic insult would decrease overall brain pathology, reduce the death of the newly formed neurons and improve long term motor and cognitive function. To conduct this study, a transient middle cerebral artery occlusion model in rats and mice will be used to examine sensorimotor and cognitive behavioral outcomes over chronic survival points. The generation of new neurons after focal ischemia will be examined in two distinct areas of the brain (subgranular and subventricular zones) that are known to

demonstrate neurogenesis after injury. Special staining approaches will be used with a novel tissue clearing technique combined with 3-dimensional reconstructions to resolve cellular responses to injury and treatment. A special transgenic mouse model (Nestin-TK-GFP) will be used to independently manipulate the degree of neurogenesis and determine a causal link to improved functional outcomes. The endpoints of the research are to determine if P7C3- A20 is a viable therapeutic strategy after experimental focal ischemia and to clarify potential mechanisms of action including enhanced neurogenesis. Importantly, if found to be effective, this neuroprotective treatment could be combined with available endovascular approaches to maximize protection and recovery.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

   Principal Investigator: Jhanelle Gray, MD

   Organization: H. Lee Moffitt Cancer Center

   Abstract of Proposed Research: Lung cancer causes more cancer deaths than breast, colon, prostate, and pancreatic cancer combined. It is an immunotherapeutically responsive cancer. Immune checkpoint inhibitors, including anti-programmed death 1/programmed death ligand (anti-PD1/PD-L1) therapies, produce improvements in median overall survival from 12 to 24 months, with some durable responses. As dramatic as these results are, less than half of patients benefit. Combination strategies that interfere with the different immunosuppressive mechanisms operational within the tumor microenvironment are of interest in lung cancer immunotherapy. This research team recently discovered that the agent nintedanib (FDA approved for idiopathic pulmonary fibrosis; approved in Europe for combination with chemotherapy in lung cancer), which blocks multiple receptors including fibroblast growth factor receptors, has the potential to be repurposed as an anti-cancer immunotherapeutic, abolishing the immunosuppressive influence of cancer-associated fibroblasts (CAFs). CAFs are the most prominent cell type in the tumor stroma and differ from normal fibroblasts as they are continuously activated. At Moffitt, they developed a technique to grow out CAF cell lines made from human lung cancer tumors. In this model, T cells are strongly inhibited in the presence of CAFs due to expression of immune checkpoints and other immunosuppressive enzymes. Based on their preclinical work and the literature, they hypothesize that targeting immunosuppressive CAFs within the tumor microenvironment in combination with immune checkpoint blockade with nivolumab may translate into better tumor control. The significance of this project is that by immune suppression blockade, they are primed to increase the immune-mediated tumor responses, identify markers that can better predict tumor shrinkage while reducing waste and toxicity (precision medicine), and enrich patient treatment algorithms and ultimately improve outcomes for patients with nonsmall cell lung cancer. With these goals in mind and based on the above, they developed a phase Ib/II clinical trial with serial blood collections and tumor biopsies to evaluate the combination of nintedanib with immune-checkpoint blockade

(nivolumab). Objective #1. To determine the safety and efficacy of nivolumab plus nintedanib in advanced lung cancer. Objective #2. To correlate key markers obtained from serial tumor biopsies and blood collections with response to treatment. Objective #3. To correlate key markers obtained from serial tumor biopsies with resistance to treatment.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

   Principal Investigator: Zhihua Jiang, PhD

   Organization: University of Florida

   Abstract of Proposed Research: This aortic disease affects 5% of the general population, with the incidence being three to five times higher in smokers than in non-smokers. Furthermore, tobacco use doubles the rate of aortic dilation and the risk of rupture. Currently, mechanisms underlying tobacco smoke-exacerbation of aortic aneurysms are poorly understood. Strategies capable of reducing or eliminating the deleterious effect of tobacco smoke on aortic aneurysm development remain unavailable. Studies for lung cancer and chronic obstructive pulmonary disease have generated rich knowledge about the impact of tobacco smoke on the biology of endothelial cells, smooth muscle cells (SMCs), and immune cells. A large body of clinical and experimental evidence supports the concept that aortic aneurysm is an inflammatory disease. Recent advances in immunology have identified two different types of inflammation, with each type of inflammation driven by a distinct subset of immune cells and cytokines. Specifically, the type 1 inflammation is governed by a subset of thymus (T) cells, called type 1 T helper or TH1 cells whereas the type 2 inflammation is dominated by TH2 cells. Under physiological conditions, the function of TH1 and TH2 cells is well-balanced to maintain tissue homeostasis. Interestingly, epidemiological investigations have shown that compared with the general population, diabetic patients are two times less likely to develop aortic aneurysms while asthma patients are at a two times greater risk of developing aortic aneurysms. A detailed characterization of the immune system of these patients has uncovered that diabetic patients have a TH1-biased immunity whereas asthma patients have a TH2-biased immunity. These studies have established a correlation of aortic aneurysm formation with the TH2-biased immune responses. As opposed to the traditional belief that tobacco smoke suppresses the immune function, emerging evidence suggests that it skews the TH1/TH2 balance toward a TH2 phenotype. This finding has provided a plausible explanation to the protective effects of diabetic condition and the detrimental effects of asthma on aortic aneurysm formation. It appears that tobacco smoke exacerbates aortic aneurysms via shifting the TH1/TH2 balance to a TH2 phenotype. In this project, the study team will use both genetic and pharmaceutical approaches to test this hypothesis.

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

   Principal Investigator: Mandip S. Sachdeva, PhD

   Organization: Florida Agriculture and Mechanical University

   Abstract of Proposed Research: An estimated one million cases of breast cancer are diagnosed annually worldwide. Of these, more than 170,000 are described as triple-negative. Triple negative breast cancer (TNBC) is defined by the lack of protein expression of estrogen receptor (ER) and progesterone receptor (PR) and the absence of human epidermal growth factor receptor 2 (HER2) protein over-expression. TNBC does not have a first line treatment. Development of an oral nanoparticle product of an already existing drug (Docetaxel which is given intravenously) in combination with another agent (Piperlongumine), which can significantly potentiate its activity in a synergistic manner against TNBC, will be of immense help to cancer patients allowing them to avoid the adverse effects involved with multiple parenteral injections and also avoid the need to go to the hospital. The ultimate goal in this proposal is to develop an oral nanoparticle capsule based formulation for the treatment of triple negative breast cancer with minimal toxicity and enhanced efficacy.

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

   Principal Investigator: Z. Hugh Fan, PhD

   Organization: University of Florida

   Abstract of Proposed Research: Cigarette smoking is the leading cause of chronic obstructive pulmonary disease (COPD). Exposure of secondhand smoke (SHS) to non-smokers also induces COPD. 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 rising popularity of e-cigarettes make the studies on SHS exposure even more important. 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 in the point of care. To address the challenge, the study team proposes to develop laminated paper-based analytical devices (LPAD) for detecting SHS exposure. LPAD will be developed by borrowing the concept from potential of hydrogen (pH) papers and pregnancy test strips. Either colorimetric reading or optical detection can be used. The LPAD devices are low-cost, easy to operate by nontechnical personnel, and manufacturable. This one-year project

aims to design and fabricate LPAD for SHS detection. The LPAD device consists of components for sample concentration and multiplexed detection, enrichment method integrated in the device will enable it to detect low abundance biomarkers. The paper-based enrichment method integrated in the device will enable it to detect low abundance biomarkers.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

James & Esther King Biomedical Research Program

APPENDIX G

FISCAL YEAR 2016-2017 ACTIVE GRANTS,
Funding Fiscal Year 2015-2016

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
6JK01	H. Lee Moffitt Cancer Center	Djeu, Julie Y.	$ 1,231,336	$ 262,792.14	$ 968,543.86	3/19/2016	2/28/2019	No	No	No
6JK02	H. Lee Moffitt Cancer Center	Drobes, David J.	$ 1,186,164	$ 227,109.94	$ 959,054.06	3/19/2016	2/28/2021	No	No	No
6JK03	University of Florida	Liao, Daiqing	$ 795,236	$ 310,834.80	$ 484,401.20	3/09/2016	2/28/2019	No	Yes	No
6JK04	Florida International University	Miguez, Maria Jose	$ 1,628,449	$ 259,904.29	$ 1,368,544.71	3/19/2016	2/28/2021	No	No	No
6JK06	H. Lee Moffitt Cancer Center	Park, Jong Y.	$ 1,231,336	$ 285,739.76	$ 945,596.24	3/21/2016	2/28/2019	No	No	No
6JK08	Florida Atlantic University	Wu, Jang-Yen	$ 1,231,336	$ 382,166.03	$ 849,169.97	3/31/2016	2/28/2019	Yes	Yes	No

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

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

   Principal Investigator: Julie Y. Djeu, PhD

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: The study team was able to consistently recover over 95% live cells from human lung cell line A549 xenografted tumors in non-obese diabetic severe combined immunodeficiency gamma (NSG) mice using the Becton Dickinson TuDORD Digestion Buffer. However, the research group continues to have difficulty finding human Natural Killer (NK) Cells within the tumor bed although they can readily be recovered from blood, liver and spleen up to three days after intravenous NK cell administration into tumor bearing mice. The group hypothesize that A549 lung tumor cells may not be expressing chemokines to attract NK cells and have earlier suggested chemokine ligand 2 (CCL2), which is lacking in A549 cells, to be the culprit. They also investigated other chemokines and have found that NK cells express high levels of chemokine receptor 1 (CX3CR1) and its ligand may be dysregulated in A549 tumor cells. They are assessing CX3CL1 production by A549 tumor cells besides CCL2 and the role they play in tumor infiltration. The second study objective is to determine if Transforming Growth Factor-beta (TGFb) produced by A549 tumor cells is involved in defective NK function *in vivo*. To answer this question, they attempted to knock down TGFb in A549 tumor cells prior to implanting them into mice and assess the penetration of NK cells into the tumor site and modulation of NK receptors/adaptor. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology was used to knock down TGFb in A549 tumor cells, confirmed by western blot analysis but under these conditions, the tumor cells rapidly died. They next turned to short hairpin ribonucleic acid (shRNA)-constructs to knock down TGFb and found that, of all four constructs used, shRNA #1 had the highest ability to deplete TGF messenger ribonucleic acid in the transfected A549 tumor cells. All the transfected tumor cells survived and grew equally well. Therefore, they used shRNA#1-transfected A549 tumor cells in NSG mice, and also injected control mice with non-transfected A549 tumor cells. Once the tumors were detectable, NK cells were administered intravenously followed by analysis of their localization in the tumor site and other organs. Knockdown of TGFb did not appear to affect NK cell localization. Similar to untransfected A549 tumor bearers, the TGFb-depleted A549 tumor bearers had comparable high levels of cell surface marker (CD56+), natural cytotoxicity receptor (NKp46+) and NK cells in blood and liver but almost none in the tumor site. Thus, loss of TGFb still did not allow NK infiltration into the tumors. Because it is impossible to study tumor infiltrating NK cells, the study team is now assessing if circulating NK cells and liver NK cells may still be affected by the tumor and will examine immunoreceptor tyrosine-based activation motif-bearing transmembrane adaptor DAP12 and micro ribonucleic acid 183 expression in these 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.

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

   Principal Investigator: David J. Drobes, PhD

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: The overall goals of this project are to develop and test an integrated (behavioral and pharmacological) smoking cessation intervention utilizing reduced nicotine content cigarettes prior to quitting, along with a targeted treatment workbook and counseling. The treatment is aimed to maximize the efficacy of very low nicotine content (VLNC) cigarettes as a smoking cessation strategy, by extinguishing nicotine-reinforced smoking behavior prior to cessation. This aim draws upon existing basic and clinical literature regarding extinction processes, and employs qualitative methods (e.g., expert/consultant review, in-depth interviews) to develop and refine the intervention. To date, the research team has progressed through several iterations of the primary treatment workbook, based on feedback from experts and outside consultants. In April 2017, a Material Transfer Agreement was executed between the Moffitt Cancer Center and the National Institute on Drug Abuse, allowing the study team to receive “Spectrum” low-nicotine research cigarettes for this project. An initial supply of the research cigarettes was received, and pilot testing with the research cigarettes and treatment workbook has been initiated. The goal is to recruit 20 pilot participants prior to the end of calendar year 2017. Based on feedback obtained during pilot testing, the workbook will be further refined prior to the onset of a larger Randomized Controlled Trial (RCT), which is expected to begin in early 2018.

   Follow On Funding: None at the time of reporting.

   Collaborations: University of South Florida - Six undergraduates have participated on the research team.

   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: Diaquing Liao, PhD

   Organization: University of Florida

   Progress Report: Understanding the biological mechanisms underlying breast cancer and identifying therapeutic interventions targeting critical breast cancer mechanism may lead to innovative treatments for advanced breast cancer. The overall goal of this grant award is to determine roles of cyclic adenosine monophosphate response element-binding protein (CBP) and the related enzyme called p300 in breast cancer and assess

whether inhibition of CBP/p300 with small molecules could be effective for treating breast cancer. Aim 1a: The goal is to assess effects of genetic downregulation of CBP/p300 using ribonucleic acid interference (RNAi) and pharmacologic inhibition of CBP/p300 on cell proliferation and survival of a panel of breast cancer cell lines derived from hormone receptor positive luminal subtype, human epidermal growth factor receptor 2 (HER2)-enriched subtype and triple-negative subtype (TNBC). The University of Florida (UF) research team has expressed short hairpin ribonucleic acids (shRNAs) targeting CBP and p300 in a number of breast cancer cell lines. Phenotypic assessment (cell survival/proliferation) of the derivatives expressing CBP/p300 shRNAs are under investigation. New tools such as the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) associated protein 9 (Cas9)) gene editing approach, are also being used to genetically inhibit CBP/p300 in order to assess roles of CBP/p300 in breast cancer biology. Aim 1b: The goal is to characterize the impact of this new pharmacologic inhibitor of p300/CBP on genome-wide chromatin occupancy and gene expression using high-throughput methods, i.e., ChIP-seq and RNA-sequencing (RNA-seq). The research team continues to validate the novel series of highly potent CBP/p300 inhibitors (EP016-EP022). Aim 2. The goal is to test whether the new CBP/p300 inhibitors can suppress tumor growth *in vivo* using breast cancer patient-derived xenograft (PDX) tumor models alone and especially in combination with standard-of-care breast cancer therapies. As stated above, they have identified a new series of CBP/p300 inhibitors (EP016 to EP022) and now these compounds are subject to further validation *in vitro*. A validated lead compound will be used for the proposed *in vivo* studies.

Follow On Funding: None at the time of reporting.

Collaborations: University of Florida-three PhD students

Journals: Wang, Y., Li, D., Luo, J., Tian, G., Zhao, L.Y. and Liao, D., Intrinsic cellular signaling mechanisms determine the sensitivity of cancer cells to virus-induced apoptosis Sci. Rep. 2016 6: 37213 doi:10.1038/srep37213

Liao, D. CBP/p300 Bromodomain mediates amyloid formation. Cell Chem. Biol. 2017 24(2):128-129. doi:10.1016/j.chembiol.2017.01.004

Patents: None at the time of reporting.

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

   Principal Investigator: Maria Jose Miguez, MD, PhD

   Organization: Florida International University

   Progress Report: Clinical studies have demonstrated that men and women are affected differently by tobacco use. Gender Differences in Participation: While enrollment of females in clinical trials is frequently limited, in this particular clinical study men and women have been enrolled at similar and even slightly higher rates. Gender and Smoking Profile: Women are younger than their male counterparts. Gender differences were not apparent in either marital status or in years of education. Participants were asked at baseline to report their frequency of smoking, preferred brand of cigarette, years of smoking and number of quit attempts. The vast majority (80%) of human

immunodeficiency virus (HIV)-infected smokers, use mentholated brands. Men and women in the study equally preferred mentholated brands (80 vs 79%). In this study, women and men smoked at the same rates (15.5 ± 11.7 vs 14.2 ±7.8 cigarettes per day). Despite smoking more cigarettes per day, females did not significantly differ in their levels of dependence, as indicated by the Fagerström test score (range = 1-10; 5.8 ± 2 vs. 5.2 ± 1.9, p=0.2) and their plasma cotinine levels (women: 192.2 ± 118 vs men: 203.2 ± 109, p=0.63). No gender differences in the number of prior attempts was observed (3.6 ± 1.8 vs. 3 ± 0.6, p=0.2). At baseline, both men and women exhibited the same readiness to quit, indicated by their scores on the Quit Ladder test. However, in the current trial, women appear to have more trouble quitting than men. Gender and Mood: Analyses indicated that women had higher T-scores in almost all the mood subscales of Profile of Mood States when compared to men, but particularly on the depression scale. The 30-day point-prevalence confirmed abstinence (PPA)-rates reached 20% for the standard arm versus 26% at 6 months. No gender or race differences that quit were evident. These rates are extraordinary when compared to the quit rates of three prior randomized trials done in people living with HIV with 6-month or longer outcomes. Different intervention approaches, such as cell-phone counselling, motivational interviewing or a combination, in these studies yielded quit rates ranging from 4% to 10%. Quit rates at the 6-month benchmark could be higher than even the 20-26%, this research verified, as a sizable proportion of HIV-infected individuals that indeed stop smoking in the quit day have started smoking because other smokers living with them, making it increasingly difficult to quit. The Fagerström Test for Nicotine Dependence (FIND) analyses reveal significant changes from baseline to 6 and 12 months for intervention targets. As expected, carbon dioxide (CO_2) significantly dropped in those who quit; and reached the desirable exhaled CO <10 ppm.

Follow On Funding: None at the time of reporting.

Collaborations: University of Miami

Journals: None at the time of reporting.

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: Moffitt Cancer Center

   Progress Report: The purpose of this study is to develop a statewide prostate cancer biobank for men of African Ancestry in Florida. During the first year, the Moffitt Cancer Center (in Tampa, Florida) obtained Institutional Review Board approval from the Florida Department of Health and received requested data from the Florida Cancer Data System (FCDS). The data included the mailing address and telephone information of over 5,000 African American prostate cancer patients who were diagnosed between 2013-2015 in Florida and their epidemiological and clinical data. With this contact information, the research team began recruiting participants for their study using invitational mail packages, and contacting patients by phone. To date, the Moffitt Cancer Center has sent out 1,724 mail packages, and is awaiting a response from 816 patients. From those who have shown interest in the study, 65 participants have signed the inform consent and

completed questionnaires, while 72 patients have yet to respond. Although language barriers and inaccurate patients' contact information received from the FCDS has caused participation delays, the research team has made significant progress towards obtaining biological samples. The plan for the duration of 2017 is to continue recruiting participants for the study, and distributing saliva collection kits to participants who have signed the informed consent and completed questionnaires. Following this aim, the research team projects to begin the deoxyribonucleic acid epigenetic experiment in Year 3 of the grant funded period.

Follow On Funding: None at the time of reporting.

Collaborations: University of Miami/Sylvester Cancer Center- Dr. Alan Pollack; and UF Health Cancer Center in Jacksonville- Dr. Shahla Masood.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

6. **Grant #6JK08:** Granulocyte colony-stimulating factor (G-CSF) gene therapy for stroke

   Principal Investigator: Jang-Yen Wu, PhD

   Organization: Florida Atlantic University

   Progress Report: Experiment 1: Bilateral carotid artery occlusion (BCAO) stroke model in mice monitored with a Laser Doppler Flowmeter (LDF). The researchers have established the mouse BCAO model for subsequent use in their analysis of the efficacy of granulocyte colony-stimulating factor (GCSF) gene therapy. Briefly, mice were anesthetized with isoflurane and then kept at a constant temperature of 36+ 1 degree C. A ventral cervical midline incision was carried out to expose the carotid arteries which were then occluded with microaneurism clips. Cerebral ischemia will be induced by 30 or 0 minutes of BCAO. At the end of the period of ischemia, the microaneurism clips were removed and the incision was sutured closed. All mice subjected to BCAO exhibited a significant reduction in regional cerebral blood flow (RCBF) to 51% pre-BCAO values and the RCBF retuned to 91% of the pre-BCAO level at the beginning of reperfusion as monitored with a LDF. Experiment 2: Neuro-protection of GCSF gene therapy in the bilateral carotid artery occlusion (BCAO) mouse stroke model as demonstrated in decrease of endoplasmic reticulum (ER) stress markers, glucose-regulated protein 78 (GRP78), phosphorylated inositol-requiring protein-1 (plRE1) and X-box binding protein 1 (XBP-1). The function of the ER is to synthesize and fold proteins destined for secretion, the cell membrane, Golgi apparatus or lysosomes. Any condition, such as ischemia, that interferes with the normal folding of proteins in the ER results in ER stress. In order to overcome the initial ER stress signal, the ER elicits a response called the Unfolded Protein Response (UPR). One of the master key regulator of UPR is GRP78. Under ER stress, GRP78 is activated which in turn activates the three ER transmembrane stress sensors: IRE1a (inositol-requiring protein-1a), PERK (protein kinase ribonucleic acid (PKR)-like ER kinase), and ATF6 (activating transcription factor 6). These three ER sensors will in turn activate respective intracellular pathways in order to alleviate ER stress. ln this experiment, they examined the effect of GCSF gene therapy on the expression of GRP78, plRE1 and XBP-1, which is one of the downstream IRE-1 pathway components. They found that GRP78 expression was greatly reduced in

BCAO animals treated with adeno-associated virus-cytomegalovirus-granulocyte colony-stimulating factor (AAV-CMV-GCSF) gene compared to those that received the control vector, AAV-CMV-GFP (green fluorescent protein) in all three brain regions, front, mid and hind brain regions by 40%, 60% and 55%, respectively. Similar results were also observed with plRE1 and XBP-1 in front and mid brain regions, but not in the hind region. Experiment 3: Neuro-protection of GCSF gene therapy in the bilateral carotid artery occlusion (BCAO) mouse stroke model as demonstrated in decrease of autophagy stress marker, Beclin-1. Stroke-induced brain injury is multifaceted and hence the mechanism of GCSF gene therapy in protecting brain from stroke-induced neuronal damage could also have multiple targets including ER, mitochondria and autophagy. Beclin-1 is a good marker for autophagy. They found that the expression of Beclin-1 is reduced in all brain regions including the front, mid and hind brain by 20, 65 and 40%, respectively. Experiment 4: Neuro-protection of GCSF gene therapy in the bilateral carotid artery occlusion (BCAO) mouse stroke model as demonstrated in decrease of dynamin-related protein 1 (DRP1), marker of mitochondrial stress, and increase of optic atrophy 1 (OPA1), marker of mitochondrial enhancer. In addition to ER and autophagy, the researchers also examined the effect of GCSF gene therapy on the function of mitochondrial activity in BCAO mice as determined by the level of mitochondrial stress marker, DRP1 and the level of mitochondrial enhancer marker, OPA1. The research team found that BCAO caused a great increase of the mitochondrial stress marker, DRP1, a six-fold increase, as shown in AW-CMV-GFP control gene treated BCAO mice compared to sham group. AW-CMV-GCSF gene treatment reduced the DRP1 level by about 20% compared to the AW-CMV-GFP control group. On the other hand, the marker of mitochondrial enhancer, OPA1, is greatly elevated, by about 45, 60 and 5%, respectively in the front, mid and hind brain compared to the CW-CMV-GFP treated group. Experiment 5: Neuro-protection of GCSF gene therapy in the bilateral carotid artery occlusion (BCAO) mouse stroke model as demonstrated in the locomotor activity test. Consistent with the Western blot analysis, it was found that AAV-CMV-GCSF gene treated BCAO mice showed protection against behavioral deficits and this was reflected in a greater activity in the locomotor activity test.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Jong, C. J., Ito, T., Prentice, H., Wu, J.-Y., and Schaffer, S.W. Role of mitochondria and endoplasmic reticulum in taurine-deficiency-mediated apoptosis. Nutrients 2017 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. Protective functions of AEURA in cell based model of stroke and Alzheimer disease. J. Neurosci. Neurol. Disord. 2017 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. 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. 2017 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. In: Lee DH., Schaffer S., Park E., Kim H. (eds.) Taurine 10. Advan. Exper. Med. Bio. 2017 975: 193-205 doi:10.1007/978-94-024-1079-2_17

Patents: The Invention Disclosure entitled “Carbamathione, S-(N,N-diethylcarbamoyl)glutathiones, as a novel agent for the treatment of stroke” was submitted to Florida Atlantic University (FAU Ref No:201708) on May 2, 2017 and the Marketing Assessment was completed on July 20, 2017.

James & Esther King Biomedical Research Program

APPENDIX H

FISCAL YEAR 2016-2017 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	$ 835,617.35	$ 1,117,382.65	5/25/2015	5/15/2019	No	No	No
5JK02	University of Miami	Salathe, Matthias	$ 1,951,531	$ 747,345.49	$ 1,204,185.51	5/25/2015	5/15/2020	No	Yes	Yes
5JK03	H. Lee Moffitt Cancer Center	Simmons, Vani N.	$ 1,904,351	$ 554,453.07	$ 1,349,897.93	5/25/2015	5/15/2020	No	No	Yes
5JK04	University of Florida	Kaye, Frederic J.	$ 1,414,858	$ 825,441.24	$ 589,416.76	5/25/2015	5/15/2018	Yes	Yes	No
5JK05	University of Florida	Ostrov, David	$ 1,464,750	$ 439,180.45	$ 1,025,569.55	5/25/2015	5/15/2018	Yes	No	No
5JK06	H. Lee Moffitt Cancer Center	Cress, Doug	$ 1,145,378	$ 728,459.81	$ 416,918.19	5/25/2015	5/15/2018	Yes	Yes	Yes

ACTIVE GRANTS FISCAL YEAR 2016-2017
(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: This research project is a multisite randomized controlled trial (RCT) being conducted at the University of Miami (UM) and Moffitt Cancer Center (MCC). The goals of the project are to 1) examine the effects of cognitive behavioral therapy (CBT) on perceived stress and depression symptoms among racially/ethnically diverse smokers, 2) test the efficacy of CBT for eliminating smoking cessation disparities, and 3) examine physiological distress as an underlying mechanism for the effects of CBT on racial/ethnic minority smokers. During the fourth quarter of the year two project period, activities at both sites focused on recruitment, enrollment, participant retention, and follow-up assessments. At UM, they also trained new research staff in study procedures, data entry and management, and intervention delivery. Given the overarching aim of examining race/ethnic differences (among African Americans, White non-Hispanics, and Hispanics) in tobacco cessation, they are continuously focusing on recruitment and engagement efforts of their study. Both research sites are on track to meet or exceed the accrual targets for African American participants. They are attracting White non-Hispanics and Hispanics to the study, however the rate of accrual is lower than anticipated. The research teams at both sites and the full study team were in regular contact to monitor recruitment and to devise new approaches. Both study sites have broadened recruitment strategies and are now delivering intervention groups at cancer center regional hospitals. Importantly, the surrounding neighborhoods have larger proportions of the demographic subgroups needed for this trial. At UM, 45 participants were screened, of which 30 (66.7%) were eligible and 15 (33.3%) were ineligible. This represents a 36.4% increase in screened participants compared to Quarter 3, Year 2 during which 33 were screened. Given that the project is on track to reach targeted sample size for recruitment and the retention rate exceeds 70%, the researchers are certain that they will have the requisite number of cases for analyzing intervention effects/follow-up assessments by the end of the study. At MCC, one individual was screened during this quarter because they have completed recruitment for the spring groups and ended the advertisement campaign in March. This one participant was eligible, randomized to an intervention group, and placed on the waitlist for Fall, 2017 groups. Two intervention groups were completed with a total of 9 participants. A total of 9 out of 9 individuals completed the treatment (100%). Three-month follow-up assessments were completed by 14 of the 19 participants (73.7%). For the six-month follow-ups, 14 out of 25 participants (56%) completed assessments, and for the twelve-month assessments 20 out of the 32 participants (62.5%) completed the assessment. Overall, the project at the MCC site is on track and will begin the next round of recruitment efforts in August.

   Follow On Funding: None at the time of reporting.

Collaborations: The University of Miami will continue to work with the H Lee Moffitt Cancer Center. There are 2 University of South Florida (USF) and 2 University of Miami graduate students, 3 USF undergraduates, and 1 Moffitt Cancer Center postdoctoral fellow receiving training and conducting research on this project.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

   Principal Investigator: Matthias Salathe, MD

   Organization: University of Miami

   Progress Report: The use of electronic (e)-cigarettes is increasing rapidly, but their lung health effects are not established. Clinical studies examining the potential long-term impact of e-cigarette use on lung health will take decades. To address this gap in knowledge, this study investigated the effects of exposure to aerosolized nicotine-free and nicotine-containing e-cigarette fluid on mouse lungs and normal human airways epithelial cells. The current aims of this study are to 1) evaluate, *in vitro* the possible acute and subacute toxic effects of electronic cigarette vapors, and 2) evaluate, *in vitro* the inflammation changes in smoking cessation with e-cigarettes and naïve users of e-cigarettes. Thus far in aim 1, the research team has performed ongoing experiments showing the effects of nicotine and cinnamaldehyde upon ion transport, airway surface liquid (ASL) volume, and mucociliary function *in vitro*. These data will also be confirmed with two different TRPA1 inhibitors and shRNA. In regard to the effects of vapor, the vapor-induced mucociliary dysfunction can be prevented with a TRPA1 inhibitor. The vapor exposure of HBECs has been proven to increase transforming growth factor β-1 (TGF- β1) and cyclooxygenase-2 (COX-2) expression as well as protein-38 mitogen-activated protein kinases phosphorylation; this fits the data collected from the human subjects. For aim 2, the researchers enrolled 54 participants, of which 10 participants are controls and 44 smokers have been assigned to e-cig use. Based on the percentage of participants that either failed to transition from tobacco smoking to e-cigs (F group) or succeed (S group), the researchers analyzed the difference between the two groups based on demographic and smoking characteristics (topography of vaping). The data suggest that a better adaptation to the device with longer inhalation times to obtain needed nicotine concentrations in the blood was associated with higher success to transition. The overall completion success rate is 24% after transition, close to the expected 25% success rate they assumed for the cohort calculation.

   Follow On Funding: Flight Attendant Medical Research Institute - $324,000

   Collaborations: Downstate University NY **(**Robert Foronjy and Pat Geraghty); Tobacco Centers of Regulatory Science (TCORS) at UNC Chapel-Hill; and National Jewish Health (Dr. Irina Petrache)

   Journals: Garcia-Arcos, I., Geraghty, P., Baumlin, N., Campos, M., Dabo, A.J., Jundi,, B., Cummins N., Eden, E., Salathe, M., and Foronjy, R. Chronic electronic cigarette

exposure in mice induces COPD in a nicotine-dependent manner. Thorax 2016 71(12): 1119-1129 doi:10.1136/thoraxjnl-2015-208039.

Shapiro, S.D. and Kaynar, A.M. Electronic cigarettes: the lesser of two evils, but how much less? Thorax 2016 71:1080-1081. doi:10.1136/thoraxjnl-2016-209273

Sailland, J., Grosche, A., Baumlin, N., Dennis, J.S., Schmid, A., Krick, S., and Salathe, M. Role of Smad3 and p38 Signaling in Cigarette Smoke-induced CFTR and BK dysfunction in Primary Human Bronchial Airway Epithelial Cells. Sci. Rep. 2017 7: 10506 doi:10.1038/s41598-017-11038-x

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 N. Simmons, PhD

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: The research team recently completed a randomized controlled trial of an extended self-help, English-language, smoking cessation intervention titled, Stop Smoking for Good. This intervention revealed high efficacy through the 24-month follow-up supporting the utility of extended self-help for promoting and maintaining tobacco abstinence. Availability of a validated Spanish-language version would enhance its public health impact by reaching the largest and fastest growing ethnic minority population of smokers. The goals of this research study are to 1) develop a culturally appropriate self-help intervention for Spanish-speaking smokers and 2) to conduct a randomized controlled trial (RCT) testing the efficacy of the intervention for promoting smoking abstinence. After completing Study 1 (Phases I, II, and III), the research team has begun Study 2, the RCT. During this phase, researchers focused on recruitment for the RCT and organizing the administration of the 6-month follow-up assessment. They have received 506 baseline questionnaires; of those 485 qualified individuals were enrolled in the study (91.6% of the proposed total sample size). In addition, 125 enrolled participants reached the 6-month follow-up time point. Out of the 125 participants, 79 have completed the assessment to date. To catch up with the proposed timeline and reach their recruitment goal, they have further expanded their recruitment efforts by working with the marketing department at Moffitt to develop ads for a public transit campaign, launched this past July, which was directed to Hispanics in the Orlando area. The project flyer was distributed to local community partners, and have highlighted their research project on a local television show targeting Hispanics. The research team plans to continue recruitment efforts for the RCT, administering the 6-month follow-up assessment, and finalizing the procedures for the 12-month biochemical verification of smoking status and 12-month follow-up assessment.

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

   Collaborations: None at the time of reporting.

   Journals: None at the time of reporting.

Patents: None at the time of reporting.

4. **Grant #5JK04:** 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: The goal of this research project is to test a new oncolytic viral agent, called myxoma virus (MYXV), as a novel treatment strategy for patients with advanced small cell lung cancer. The research team has continued to make steady progress on each of the proposed research aims. For projects 1 and 2, they have efficient cell killing using an expanded number of human and mouse lung cancer cell lines with both the parental myxoma virus and a modified viral backbone that exhibits enhanced cell killing and reduced toxicity. The investigators have also collected fresh small cell lung cancer biopsy samples from newly diagnosed patients using an IRB approved protocol and demonstrated efficient infection, viral replication, and tumor cell death with no effect on normal tissues. The investigators recognized that clinically meaningful anti-cancer treatment using oncolytic ablative therapy will require concurrent synergy with other cytotoxic and immune activation strategies. Therefore, they have extended their research to test the efficacy of myxoma virus therapy concurrent with delivery of immunotherapy using anti-programmed cell death protein 1 (anti-PD1 or anti-CTLA4) antibody in a mouse syngeneic immunocompetent small cell lung cancer model. They have demonstrated enhanced host immune cell infiltration of tumor following myoma virus delivered either intrapulmonary or by direct intratumoral injections. They have also tested all possible scheduling combinations of myxoma virus plus immunotherapy to help guide the design of a future phase 1 clinical trial in patients. Finally, the investigators also completed a survival analysis of the immunocompetent small cell lung cancer genetically engineered mouse model. One hundred percent of the genetically engineered mice developed small cell lung cancer by four months of age and die of this disease. In this model, they observed statistically significant prolongation of survival in mice treated with intrapulmonary delivery of myxoma virus and marked prolongation of survival in mice treated with combined myxoma virus with low dose cisplatin chemotherapy as compared with mice treated with chemotherapy alone or a buffered saline control solution. The researchers have also confirmed the ability of MYXV to efficiently undergo viral replication in human small cell lung tumor cells by direct imaging using electron microscopy. The ultimate goals of this preclinical data are to provide the rationale for a proposed new phase 1 clinical trial testing this new oncolytic virotherapy in lung cancer.

   Follow On Funding: None at the time of reporting.

   Collaborations: University of Florida Departments of: Medicine, Molecular Genetics and Microbiology, and Anatomy and Cell Biology, College of Medicine (Daniel Shabashvilli, Patrick Kellish graduate students and undergraduate students); DNAtrix company (Houston, TX); Florida Biologix/BrammerBio (Alachua, FL); and Moffitt Cancer Center.

Journals: Shabashvilli D, Kellish P, Rahman M, Nawab A, Guijarro M, Zhang M, Cao C, Moussatche M, Renihard M, Jantz M, Mehta H, McFadden G, Kaye FJ, Zajac-Kaye M. Oncolytic Myxoma Virotherapy For Small Cell Lung Cancer, 2017

Patents: Grant McFadden (a co-PI on this study) has a prior invention related to MYXV for oncolytic cancer therapy which he has licensed to DNAtrix, Houston, TX. Filed by UF on March 17, 2014. PCT US2014/030143

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

   Principal Investigator: David A. Ostrov, PhD

   Organization: University of Florida

   Progress Report: The alpha-1-antitrypsin (AAT) deficiency-associated lung disease (chronic obstructive pulmonary disease and emphysema) is caused by reduced levels of AAT in the serum resulting in chronic pulmonary tissue damage by neutrophil elastase. Smoking is the single most important risk factor. Currently, there is no effective treatment for both lung and liver diseases associated with AAT deficiency. This project focus will be to translate novel technology from recently discovered small molecules, into therapeutic drugs for clinical application. During the period of this grant, the researchers aim to 1) identify more effective lead compounds through an in silico molecular docking approach and functional cell culture system, 2) improve the efficacy of the lead compounds on interfering with ATZ polymer formation and ATZ secretion through computer-assisted molecular optimization, and 3) to test the effectiveness of the lead compounds in transgenic animal model. In aim 1, the researchers have identified two active candidate compounds by high throughput molecular docking (4',5-Methylenedioxy-2-nitrocinnamic acid, compound 1, and 5-methyl-3-[(6-nitro-2H-1,3-benzodioxol-5-yl) methylidene] oxolan-2-one, compound 3) that are being tested *in vivo*. Recently, they have identified compounds more effective in reducing the intracellular concentration of ATZ in hepatocytes than compounds 1 and 3 using their functional cell culture system and screening the compounds via molecular docking. In aim 2, the orientations of the active lead compounds with structural pockets provided the basis for optimization to improve intermolecular contacts with both the ATZ (Protein Data Bank code 1MQB) and the native form (Protein Data Bank 1QLP). These data lead them to discover that the active compound which binds the native active form is predicted to contact a separate structural pocket in the cleaved form. To accomplish the third aim, the researchers tested compounds 1 and 3 in transgenic mice expressing human ATZ. Based on these data, they measured the effects of treatment with small molecule candidates at different timepoints using multiple readouts and are planning to continue their endeavors throughout the grant period.

   Follow On Funding: None at the time of reporting.

   Collaborations: University of Florida (Dr. Brantly Dr. Ostrov, Dr. Chengguo Xing, Dr. Hati); and Torrey Pines Institute.

   Journals: None at the time of reporting.

Patents: Dr. Chen Liu and Dr. David Ostrov from the University of Florida submitted a patent for publication on March 26, 2009. PCT/US2007/022717. Pub #: WO2008143633 A3

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

   Principal Investigator: Doug Cress, PhD

   Organization: H. Lee Moffitt Cancer Center

   Progress Report: Adenocarcinoma is more likely to affect younger people, women and non-smokers. Early-stage lung adenocarcinoma patients are treated first with surgery to remove the affected lobe of the lung. At this point, physicians must choose if that patient should then receive adjuvant chemotherapy (ACT) to increase the chance of long-term survival. This choice is usually made based on the tumor stage. Stage I patients do not get ACT and Stage II patients do. The purpose of this study was to develop a NanoString-based molecular assay that would measure gene-expression in the normal pathology blocks from the tumors of early-stage patients and identify which patients are likely to benefit from ACT and which do not. This study examined thousands of patients and successfully developed and published an assay that is ready to be developed into the clinical environment. The team found that some Stage I patients would benefit from ACT in terms of living long and the test can predict which patients they are. Surveys with oncologists indicate that most would use the test to give Stage I patients ACT. The team finds that many Stage II patients do not benefit from ACT and their test can predict which patients they are. Presently, oncologists are unlikely to give ACT in Stage II patients, regardless of the test results. The study team's intention for the future will be to incorporate this test into practice at Moffit Cancer Center and integrate it into a decision-support tool for lung adenocarcinoma Stage I patients.

   Follow On Funding: National Cancer Institute - $110,601 and $301,537

   Collaborations: The University of South Florida (Cancer Biology Ph.D. Program, Ph.D. Candidate, Nicholas Gimbrone, and Undergraduate Program, Jason I. Rivera, Christian Lopez and 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. Early2 factor (E2F) deregulation is a prognostic and predictive biomarker in lung adenocarcinoma. Oncotarget 2016 7(50): 82254-82265 doi:10.18632/oncotarget.12672

   Chen, D-T., Huang, P-Y., Lin, H-Y., Haura, E.B., Antonia, S.J., Cress, D.C., and Gray, J.E. Strategies for power calculations in predicative biomarker studies in survival data. Oncotarget 2017 7(49): 80373-80381 doi:10.18632/oncotarget.12124

   Chen, L., Engel, B.E., Welsh, E.A., Yoder, S.J., Brantley, S.G., Chen, D.T., Beg, A.A., Cao, C., Kaye, F.J., Haura, E.B., Schabath, M.B. and Cress, W.D. A sensitive NanoString-based assay to score STK11 (LKB1) pathway disruption in lung adenocarcinoma. J. Thorac. Oncol. 2016 11(6): 838-849 doi:10.1016/j.jtho.2016.02.009

Gimbrone, N.T., Sarcar, B., Gordian, E.R., Rivera, J.I., Lopez, C., Yoder, S.J., Teer, J.K., Welsh, E.A., Chiaporri, A.A., Schabath, M.B., Reuther, G.W., Dutil, J., Garcia, M., Ventosilla-Villanueva, R., Vera-Valdivia, L., Yabar-Berrocal, A., Motta-Guerrero, R., Santiago-Cardona, P.G., Munoz-Antonia, T. and Cress, W.D. Brief Report: Somatic Mutations and Ancestry Markers in Hispanic Lung Cancer Patients. J. Thorac. Oncol. 2017 (In Press) doi:10.1016/j.jtho.2017.08.019

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

Patents: Biomarkers and methods for predicting benefit of adjuvant chemotherapy WO 2014121177 A1 Inventors: Douglas Cress, Dung-Tsa Chen Assignee: H. Lee Moffitt Cancer Center and Research Institute, Inc. Filed February 1, 2013 and assigned U.S. Serial No. 61/759,763, File internationally February 3, 2014 (PCT patent application)

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APPENDIX I

FISCAL YEAR 2016-2017 ACTIVE 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 A.	$ 1,600,000	$ 1,101,514.96	$ 498,485.04	6/26/2014	12/31/2017	No	Yes	Yes
4KB17	H. Lee Moffitt Cancer Center	Antonia, Scott	$ 1,600,000	$ 1,254,661.10	$ 345,338.90	6/26/2014	12/31/2017	No	Yes	Yes

ACTIVE GRANTS FISCAL YEAR 2016-2017
(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— The 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. Pragmatic Trials and Implementation Science Minority Education Program (PTIS-MEP) Core— The Minority Education Program (MEP) scholars attended the OneFlorida Annual Stakeholder Meeting in Gainesville on January 26, 2017. Each scholar prepared and presented a poster on his or her MEP project. Trainees' projects are well underway, including plans for publication or presentations. Tobacco Use Assessment and Cessation Referral in Pediatric Cancer— Since implementing the 6As in Pediatric Primary Care and Tobacco Use Pediatric Patient Registry in February 2017, the research staff set a goal to recruit 200 teens and their parents across the three projects. Thus far, for the 6As project, the research team has screened 52 participants and made 4 referrals to Area Health Education Centers (AHEC). Tobacco Use Assessment and Cessation Referral in Adult Primary Care-- 66 participants have enrolled in the study. Implementing the 6As in Pediatric Primary Care—58 participants have enrolled in the study and we are continuing to enroll patients. In addition, five teens have been added to the patient registry. Clinicians and clinic managers have completed five surveys in total and made 10 referrals to Area Health Education Centers.

   Follow On Funding: Patient-Centered Outcomes Research Institute - $1,599,999.99; National Cancer Institute - $407,050; Agency for Healthcare Research and Quality - $2,638,534

   Collaborations: University of Miami; Florida State University; Florida Agricultural and Mechanical University; and Edward Waters College; and Bethune-Cookman University.

   Journals: Hicks, A., Hanna, J., Welch, D., Brochhausen, M., and Hogan, W.R. The Ontology of Medically Related Social Entities (OMRSE): Recent Developments. Journal of Biomedical Semantics. July 2016. 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. Use of Electronic Cigarettes Among Cancer Survivors in the U.S. American J. of Preventive Medicine. Nov. 2016. 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 Medicine. Feb. 2016. Doi: 10.1016/j.amepre.2016.10.015

Salloum, R.G., Haider, M.R., Barnett, T.E., Guo, Y., Getz K.R., Thrasher, J.F., Maziak, W. Waterpipe Tobacco Smoking and Susceptibility to Cigarette Smoking Among Young Adults in the United States, 2012–2013. J. Prev. Chronic Dis. Feb 2016. DOI:10.5888/pcd13.150505

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 are to expand and improve existing infrastructure to support lung cancer screening research at the Moffitt Cancer Center. The objectives are 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. They are in the process of developing the best tools to publicize lung cancer screening education. This year, the CT Lung Screening and Surveillance program coordinator has screened 77 new patients for eligibility. Of these, 57 patients (74%) met required National Comprehensive Cancer Network (NCCN) guidelines and commercial insurance screening standards. Recruitment efforts continue at all Moffitt Cancer Center locations. To date, they have consented 279 lung cancer screening patients and have curated 732 CT images for Radiomic analyses. Additionally, recruitment efforts have expanded to include incidental pulmonary nodule patients. They have identified 2,662 incidental pulmonary nodule patients, of which 40 have been consented. For Radiomic analysis, 1,210 CT images have been curated. There are more studies using the tool for data analysis resulting in more realistic stress testing. Concurrently a new clinic-ready build has been completed which offers more robust, cross modality analysis tools and solutions to many of their previous bandwidth constraints. To date, all of the follow-up time-points of the pilot study, including the 1, 3, 6, and 9-month assessments, have been completed. In an effort to augment findings from the pilot feasibility trial, participants from the trial are being recontacted for in-depth qualitative interviews. The goal of the interviews is to further evaluate the new intervention and participants' experience during and after the Low-dose computed tomography (LDCT) screening. Interviews are currently in progress with 5 interviews completed to date. The R01 for a fully-powered Randomized Clinical Trial (RCT) that National Cancer Institute (NCI) was considering for funding ultimately was not funded. They are considering other funding options to test the newly developed smoking cessation intervention for LDCT patients.

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

   Collaborations: Vanderbilt University (Pierre Massion)

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

Simmons, V.N., Gray, J.E., Schabath, M.B., Wilson, L.E., and Quinn, G.P. High-risk community and primary care providers knowledge about and barriers to low-dose computed tomography lung cancer screening. Lung Cancer 2017 106: 42-29 doi:10.1016/j.lungcan.2017.01.012 PMID: 28285693.

Shafiq-ul-Hassan, M., Zhang, G.G., Latifi, K., Ullah, G., Hunt, D.C., Balagurunathan, Y., Abdalah, M.A., Schabath, M.B., Goldgof, D.G., Mackin, D., Court, L.E., Gillies, R.J. and Moros, E.G. Intrinsic dependencies of CT radiomic features on voxel size and number of gray levels. Med. Phys. 2017 44: 1050-62 doi:10.1002/mp.12123

O'Connor, J.P., Aboagye, E.O., Adams, J.E., Aerts, H.J., Barrington, S.F., Beer, A.J., et al. Imaging biomarker roadmap for cancer studies. Nat. Rev. Clin. Oncol. 2017 14(3):169-86 doi:10.1038/nrclinonc.2016.162

Liu, Y., Balagurunathan, Y., Atwater, T., Antic, S., Li, Q., Walker, R.C., Smith, G., Massion, P.P., Schabath, M.B. and Gillies, R.J. Radiological Image Traits Predictive of Cancer Status in Pulmonary Nodules. Clin. Canc. Res. 2017 23:1442-1449 doi:10.1158/1078-0432.CCR-15-3102

Patents: None at the time of reporting.

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APPENDIX J

FISCAL YEAR 2016-2017 COMPLETED GRANTS,
Funding Fiscal Year 2015-2016

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
6JK05	University of South Florida	Mohapatra, Shyam S.	$ 100,000	$ 99,915.75	$ 84.25	3/31/2016	8/31/2016	Yes	Yes	No
6JK07	Sanford Burnham Medical Research Institute	Rastinejad, Fraydoon	$ 100,000	$ 100,000	$ 0	3/07/2016	8/31/2016	No	Yes	No

COMPLETED GRANTS FISCAL YEAR 2016-2017

(Funding Year 2015-2016)

1. **Grant #6JK05:** Microfluidic-Acoustic Biosensing-Multicell Tumoroid (MABMCT) Platform Targeting TME

   Principal Investigator: Shyam S. Mohapatra, PhD

   Organization: University of South Florida

   Progress Report: This research funding focused on developing and testing a highly innovative and robust 3D multi-cell tumoroid (MCT) coupled with microfluidic-acoustic biosensing-multicell tumoroid (MABMCT) platform that permits perfused tumoroid culture and biosensing. This technology is expected to provide an *in vitro* tumor model that fully

   reflects human *in vivo* tumors. There were two aims. Aim 1 focused on the development of in-house MABMCT prototypes, including fabrication of acoustic electrodes for the MABMCT system and real-time sensing of biomarkers in single cell tumoroids (SCTs). Aim 2 focused on developing microfluidic systems for drug penetration studies in tumoroids. Toward these goals in the available 5-month period, a number of single chamber unit and multi-chamber (3 to 4 chamber) perfused units were designed and tested. Also, the research team developed drug penetration studies demonstrating that the tumoroids significantly differ from traditional 2D cultures and they are similar to cultures using the *in vivo* tumors.

   Follow On Funding: None at the time of reporting.

   Collaborations: None at the time of reporting.

   Journals: Nair, R.R., Padhee, S., Green, R., Das, T., Howell, M., Mohapatra, S.S. and Mohapatra, S. Three- and four-dimensional spheroid and FiSS Tumoroid Cultures: Platforms for Drug Discovery and Development, and Translational Research. Critic. Rev.™ Therap. Drug Carrier Sys. 201734(3): 185-208 doi:10.1615/CritRevTherDrugCarrierSyst.2017018042

   Patents: System and Method for measuring cell Viability cell growth-PCT Filed by USF PCT/US2016/018762, (Filed by USF: 5/18/2016)
   Breast Cancer Drug Screening Utilizing Tumoroids Grown on a Novel Nanofiber-Scaffold Platform (Filed by USF: 16A038PR)
   Screening of clinically approved anti-cancer drugs for use in colorectal

   adenocarcinoma (Filed by USF: 16A035 PR)

2. **Grant #6JK07:** Molecular Characterization of Nrf2 as a Drug Target in Cancer Therapeutic Resistance

   Principal Investigator: Fraydoon Rastinejad, PhD

   Organization: Sanford Burnham Presby. Medical Discovery Institute

Progress Report: The research team designed and implemented studies to understand the architecture of the nuclear factor (erythroid-derived 2)-small musculoaponeurotic fibrosarcoma (NRF2-MafG) and its specific interactions with a specific NRF2 inhibitor, ML385. They expanded the crystallization platform for NRF2-MafG-DNA (deoxyribonucleic acid) by generating multi-milligram quantities of the NRF2-MafG heterodimer from *Escherichia coli* expression. They also designed and implemented a novel biophysical assay to detect NRF2-MafG-DNA interactions with ML385. They were able to measure quantitatively, the equilibrium binding constant affinity (Kd) of the heterodimer with this compound, and assess the cellular efficacy of the compound at inhibiting transcriptional activity of NRF2 (half maximal inhibitory concentration). The crystal optimization has been a significant challenge but preliminary diffraction shows promise for further improvement through variation of cryogenic conditions. The small crystalline specimens obtained were soaked with ML385, and extensive co-crystallization screens were also initiated in parallel. Deletion constructs of NRF2 were designed to assess the precise site of ML385 binding. The study team discovered and characterized this novel NRF2 inhibitor from a high-throughput screen and showed its binding and mechanism of action with respect to NRF2. This compound potently inhibits the key downstream target genes of NRF2 in cellular assays. In clonogenic assays, and when used in combination with platinum-based drugs, doxorubicin or taxol, ML385 clearly enhances the cytotoxicity in non-small cell lung cancer (NSCLC) cells. ML385 shows particular selectivity for NSCLC cells that simultaneously harbor the Kelch-like ECH Associated Protein 1 mutation that causes the up-regulation of NRF2 activity. In preclinical models of NSCLC with gain of NRF2 function, ML385 in combination with carboplatin showed significant antitumor activity. Therefore, they propose that ML385 is a possible pre-clinical candidate for further development and testing in the context of some lung cancers.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Singh, A., Venkannagari, S., Oh, K.H., Zhang, Y-Q., Rohde, J.M., Liu, L., Nimmagadda, S., Sudini, k., Brimacombe, K.R., Gajghate, S., Ma, J., Wang, A. Xu, X., Shahane, S.A., Xia, M., Woo, J., Mensah, G.A., Wang, Z., Ferrer, M., Gabrielson, E., Li, Z., Rastineiad, F., Shen, M., Boxer, M.B. and Biswal, S. Small Molecule Inhibitor of NRF2 Selectively Intervenes Therapeutic Resistance in KEAP1-Deficient NSCLC Tumors. ACS Chem. Biol. 2016 11:3214-3225 doi:10.1021/acschembio.6b00651

Patents: None at the time of reporting.

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APPENDIX K

FISCAL YEAR 2016-2017 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
4KB09	Florida State University	Ma, Teng	$ 400,000	$ 399,597.58	$ 402.42	12/16/2013	11/30/2016	Yes	Yes	Yes

CLOSED GRANTS FISCAL YEAR 2016-2017

(Funding Year 2013-2014)

1. **Grant #4KB09:** Translation of Human Mesenchymal Stem Cell Therapy for Stroke Treatment: Bioreactor Expansion, Functional Activation, And Intranasal Delivery

   Principal Investigator: Teng Ma, PhD

   Organization: Florida State University

   Progress Report: The research team expanded bone marrow-derived human mesenchymal stem cells (hMSCs) in microcarrier bioreactor and determined cell doubling time in the microcarrier bioreactor. They coated microcarriers with thermal responsive polymer poly(N-isopropylacrylamide) (PNIPAM) and demonstrated cell detachment from the thermal responsive microcarriers. Animal protocol was approved and transplantation of aggregate-derived hMSCs was performed immediately after stroke induction in rats. Migration of transplanted hMSC was determined by magnetic resonance imaging (MRI) at the National High Magnetic Field Laboratory at Florida State University. The secretory profiles of aggregate-derived hMSCs were determined and the mechanism of functional activation determined. The formation of three-dimensional hMSC aggregates in the single-use, wave-rocking bioreactor (Wave Bioreactor™) was demonstrated. This is the first time that hMSC aggregates were produced in the Wave Bioreactor™. Migration and *in vivo* permanence of the transplanted hMSCs were quantified. The aggregate-derived hMSCs have higher permanence in the stroke lesion. Different types of microcarriers were tested in the bioreactor to determine the optimal cell expansion kinetics. The secretory profiles of hMSC aggregates were characterized using both enzyme-linked immunosorbent assay and reverse transcription polymerase chain reaction. Transplantation of aggregate-derived hMSCs were performed and MRI analyses were carried out. Thermal responsive microcarriers were developed and tested in the spinner flask and PBS Biotech bioreactors. MRI and behavior analyses of rats were performed. The results show significant improvement of stroke lesion recovery in rats treated with aggregate-derive hMSCs by ^{23}Na-magnetic resonance but not ^{1}H images. Significant improvement in stroke rats post-hMSC transplantation was observed in open field, elevated maze plus, and forelimb asymmetry cylinder tests. Exosome from hMSC culture were collected and MRI analysis was carried out. No significant improvement in behavior tests in the stroke rats' post-transplantation of the exosome was observed.

   Follow On Funding: National Institute of Health - $31,406 and $360,874

   Collaborations: None at the time of reporting.

   Journals: Sart, S., Ma, T., Lui, Y. and Li, Y. Microenvironment regulation of pluripotent stem cell-derived neural progenitor aggregates by human mesenchymal stem cell secretome. Tissue Engineer. Part A 2014 20(19-20): 2666-2679 doi:10.1089/ten.tea.2013.0437

   Ma, T. Acellular biomaterials in mesenchymal stem cell-mediated endogenous tissue regeneration J. Mater. Chem. B 2014 2(1): 31-35 doi:10.1039/C3TB21369B

Sart, S., Ma, T., and Li, Y. Extracellular matrices decellularized from embryonic stem cells maintained their structure and signaling specificity. Tissue Engineer. Part A 2013 20(1-2): 54-66 doi:10.1089/ten.tea.2012.0690

Sart, S., Tsai, A.C., Li, Y., and Ma, T. Three-dimensional aggregates of mesenchymal stem cells: cellular mechanisms, biological properties, and applications. Tissue Engineer. Part B Review 2013 20(5): 365-380 doi:10.1089/ten.teb.2013.0537

Munoz, N., Kim, J., Liu, Y., Logan, T.M., and Ma, T. Gas chromatography-mass spectrometry analysis of human mesenchymal stem cell metabolism during proliferation and osteogenic differentiation under different oxygen tensions.
J. Biotechnol. 2014 169: 95-102 doi:10.1016/j.jbiotec.2013.11.010

Li, Y., Xu, C. and Ma. T. In vitro organogenesis from pluripotent stem cells. Organogenesis 2014 10(2): 159-163 doi:10.4161/org.28918

Sart, S., Ma, T. and Li, Y. Preconditioning stem cells for in vivo delivery. BioRes. Open Access 2014 3(4): 137-149 doi:10.1089/biores.2014.0012

Sellgren, K.L. and Ma, T. Effects of flow configuration on bone tissue engineering using human mesenchymal stem cells in 3D chitosan composite scaffolds. J. Biomed. Mater. Res. Part A 2015 103(8): 2509-2520 doi:10.1002/jbm.a.35386

Liu, Y., Muñoz, N., Bunnell, B.A., Logan, T.M. and Ma, T. Density-dependent metabolic heterogeneity in human mesenchymal stem cells. Stem Cells 2015 33(11): 3368-3381 doi:10.1002/stem.2097

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Wang, Z., Xia, J., Yan, Y., Tsai, A-C., Li, Y., Ma, T. and Guan, J. Facile functionalization and assembly of live cells with microcontact-printed polymeric biomaterials. Acta Biomaterialia. 2015 11: 80-87 doi:10.1016/j.actbio.2014.10.006

Sart, S., Bejarano, F.C., Baird, M.A., Yan, Y., Rosenberg, J.T., Ma, T., Grant, S.C. and Li, Y. Intracellular labeling of mouse embryonic stem cell-derived neural progenitor aggregates with micron-sized particles of iron oxide. Cytotherapy 2015 17(1): 98-111 doi:10.1016/j.jcyt.2014.09.008

Ma, T., Tsai, A-C. and Liu, Y. Biomanufacturing of human mesenchymal stem cells in cell therapy: Influence of microenvironment on scalable expansion in bioreactors. Biochem. Engineer. J. 2016 108: 44-50 doi:10.1016/j.bej.2015.07.014

Tsai, A-C., Liu, Y. and Ma, T. Expansion of human mesenchymal stem cells in fibrous bed bioreactor. Biochem. Engineer. J. 2016 108: 51-57 doi:10.1016/j.bej.2015.09.002

Sart, S., Yan, Y., Li, Y., Lochner, E., Zeng, C., Ma, T. and Li, Y. Crosslinking of extracellular matrix scaffolds derived from pluripotent stem cell aggregates modulates neural differentiation. Acta Biomaterialia. 2016 30: 222-232 doi:10.1016/j.actbio.2015.11.016

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Patents: Materials and methods for expansion of stem cells. Inventor: Ma, Teng, US Patent Application No. 61/946,415, Filed February 28, 2014

Extracellular matrix derived from stem cells and methods for production. Inventors: Li, Yan; Ma, Teng; and Sebastian Sart, Application No. PCT/US2014/011518, Filed January 14, 2014

Zika Research Grant Initiative

APPENDIX L

FISCAL YEAR 2016-2017 NEWLY AWARDED ACTIVE GRANTS

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	$ 112,795.21	$ 1,028,789.79	3/01/2017	3/31/2020	No	No	No
7ZK02	Florida State University	Holmes, Eric	$ 1,113,645	$ 0	$ 1,113,645	3/09/2017	3/31/2020	No	No	No
7ZK03	University of Miami	Strbo, Natasa	$ 981,901	$ 87,673.53	$ 894,227.47	3/01/2017	3/31/2020	No	No	No
7ZK04	University of Central Florida	Huo, Qun	$ 199,280	$ 25,516.19	$ 173,763.81	3/08/2017	9/30/2018	No	Yes	No
7ZK05	University of Central Florida	Chumbimuni - Torres, Karin	$ 198,875	$ 36,886.35	$ 161,988.65	3/08/2017	9/30/2018	No	No	No
7ZK06	Florida State University	Megraw, Tim	$ 856,750	$ 43,227.91	$ 813,522.09	3/08/2017	3/31/2020	No	No	No
7ZK07	The Scripps Research Institute	Choe, Hyeryun	$ 199,280	$ 27,396.48	$ 171,883.52	3/08/2017	3/31/2018	No	No	No
7ZK08	University of Miami	Bandstra, Emmalee S.	$ 1,989,654	$ 18,454.20	$ 1,971,199.80	3/09/2017	3/31/2020	No	No	No
7ZK09	Florida International University	El-Hage, Nazira	$ 1,984,536	$ 67,239.49	$ 1,917,296.51	3/09/2017	3/31/2020	No	No	No
7ZK10	Florida Atlantic University	Asghar, Waseem	$ 199,280	$ 27,902.91	$ 171,377.09	3/08/2017	9/30/2018	No	No	No
7ZK11	University of Miami	Deo, Sapna K.	$ 199,280	$ 55,805.96	$ 143,474.04	3/08/2017	3/31/2018	No	No	No
7ZK12	University of Florida	Nguyen, Cuong	$ 868,744	$ 79,173.41	$ 789,570.59	3/08/2017	3/31/2020	No	Yes	No
7ZK13	Nova Southeastern University	Beljanski, Vladimir	$ 198,886	$ 59,853.42	$ 139,032.58	3/08/2017	3/31/2018	No	No	No
7ZK14	University of Miami	Saigal, Gaurav	$ 1,141,457	$ 30,521.74	$ 1,110,935.26	3/09/2017	3/31/2020	No	No	No

7ZK15	University of Florida	Alto, Barry W.	$ 199,144	$ 17,897.30	$ 181,246.70	3/14/2017	3/31/2018	No	No	No
7ZK16	Florida State University	Meckes, David G.	$ 199,280	$ 39,019.59	$ 160,260.41	3/01/2017	3/31/2018	No	No	No
7ZK17	University of Central Florida	Parks, Griffith D.	$ 500,408	$ 22,849.69	$ 477,558.31	3/01/2017	3/31/2019	No	No	No
7ZK18	University of Florida	Morris Jr., John Glenn	$ 198,812	$ 32,819.28	$ 165,992.72	3/01/2017	3/31/2018	No	No	No
7ZK19	University of South Florida	Casale, Thomas B.	$ 1,117,413	$ 83,193.99	$ 1,034,219.01	3/02/2017	3/31/2020	No	No	No
7ZK20	University of Miami	Younis, Ramzi T.	$ 1,140,125	$ 39,829.90	$ 1,100,295.10	3/09/2017	3/31/2020	No	Yes	No
7ZK21	University of Miami	Barber, Glen N.	$ 1,141,582	$ 29,808.03	$ 1,111,773.97	3/09/2017	3/31/2020	Yes	Yes	No
7ZK22	University of Florida	Fan, Hugh Z.	$ 515,377	$ 34,845.16	$ 480,531.84	3/01/2017	3/31/2020	No	Yes	Yes
7ZK23	University of South Florida	Lockwood, Charles J.	$ 1,141,582	$ 90,252.36	$ 1,051,329.64	3/07/2017	3/31/2020	No	No	No
7ZK24	University of Miami	Martinez, Claudia	$ 963,109	$ 53,546.72	$ 909,562.28	3/08/2017	3/31/2020	No	No	No
7ZK25	Florida International University	DeGennaro, Matthew J.	$ 198,468	$ 19,781.21	$ 178,686.79	3/08/2017	3/31/2018	No	No	No
7ZK26	University of Miami	Gonzalez, Ivan A.	$ 1,989,654	$ 82,139.35	$ 1,907,514.65	3/09/2017	3/31/2020	No	No	No
7ZK27	University of Miami	Stevenson, Mario	$ 1,141,582	$ 8,300.78	$ 1,133,281.22	3/07/2017	3/31/2020	No	No	No
7ZK28	University of Miami	Dhar, Shanta	$ 1,141,582	$ 96,046.11	$ 1,045,535.89	3/07/2017	3/31/2020	No	No	No
7ZK29	H. Lee Moffitt Cancer Center	Monteiro, NA Alvaro	$ 199,280	$ 46,253.41	$ 153,026.59	3/08/2017	3/31/2018	No	No	No
7ZK30	University of Florida	Brown, Ashley N.	$ 1,140,922	$ 75,322.57	$ 1,065,599.43	3/07/2017	3/31/2020	No	Yes	No
7ZK31	University of Miami	Sharkey, Mark E.	$ 199,273	$ 9,879.83	$ 189,393.17	3/08/2017	3/31/2018	No	No	No
7ZK32	University of Central Florida	Bagci, Ulas	$ 199,254	$ 5,852.20	$ 193,401.80	3/09/2017	3/31/2018	No	No	No
7ZK33	University of Central Florida	Gerasimova, Yulia	$ 200,000	$ 32,543.34	$ 167,456.66	3/08/2017	9/30/2018	No	No	No
7ZK34	University of South Florida	Teng, Michael N.	$ 200,000	$ 43,933.11	$ 156,066.89	2/28/2017	9/30/2018	No	No	No

NEWLY AWARDED GRANTS FISCAL YEAR 2016-2017

(Funding Year 2016-2017)

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

   Principal Investigator: Sylvia Daunert, PhD

   Organization: University of Miami

   Abstract of Proposed Research: Very recently, Miami has emerged as the US epicenter of the now-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 raises enormous concern as to the potential impact of ZIKV. The urgent needs are to identify individuals at risk for ZIKV infection (pregnant women and couples wishing to become pregnant). The goals of this project are 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. Fortunately, the researchers are in a unique position to rapidly develop these diagnostics. In aim 1, they plan to refine and test their ZIKV diagnostic assays for both the presence of the ZIKV virus in the acute phase and serological reactivity in the convalescent phase. For aim 2, they plan to develop this assay into a point of care (PoC) method for rapid detection of ZIKV infection that can be carried out in the doctor's office in 30 minutes or less. During this reporting period, they have complied immunoassay results in urine and determined parameters for the assay. They are also making progress toward aim 2 by preparing the PoC lateral flow assay (LFA) sticks and working on optimizing their assay protocols. With the plan to continue optimization before running their proposed experiments, the research team anticipates that this project will lead to rapid tests for ZIKV infection and previous ZIKV exposure.
   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

   Abstract of Proposed Research: The purpose of this project is to explore the potential of 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 have not been conducted on humans and the intent is to determine if the approved 2 gram/day dose would be adequate to achieve a blood level in the 3- to 10 micromolar range. Should this level be achieved, it would

be several multiples of the concentration shown to inhibit Zika replication *in vitro* and would give justification that a therapeutic dose could be achieved. Given the significant public health threat posed by Zika, especially in pregnant women, the availability of an easily adapted marketed drug for Zika therapeutics represents a major opportunity. In particular, use of an off-the-shelf therapeutic could make a rapid and critical impact and buy important time. In aim 1, the researchers plan to conduct a human pharmacokinetic analysis of single and multiple doses of 2 grams of Niclocide. A fed/fasted PK analysis will be conducted on 12-15 normal individuals in a cross-over study. These individuals will be given 3 doses of Niclocide at 24 hour intervals. Blood levels of niclosamide will be determined at specified time points and PK parameters will be determined based on the data collected. In aim 2, they plan to perform 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 will be 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 will be determined daily. The results from this clinical test will provide important information concerning the potential of niclosamide (Niclocide) to treat Zika infections and justify larger clinical studies required for regulatory approval of therapeutic treatments for Zika infected individuals.

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

   Abstract of Proposed Research: The Zika virus (ZIKV) is a single strand positive sense RNA virus that belongs to the Flaviviridae family. ZIKV infection is mostly asymptomatic and can be associated in some cases with a self-limiting mild illness. However, infection in pregnant women is of major concern, as it is linked to catastrophic fetal abnormalities including microcephaly. The placenta acts as a barrier against infections, due to multiple unique structural, cellular, and immune properties. The detrimental effects of congenital viruses, including ZIKV, on pregnancy and fetal outcomes occur in part because of impaired placental function and profound pathological changes have been observed in ZIKV-infected placentas. Thus, the researchers' hypothesis is that induction of appropriate ZIKV vaccine-induced immune responses in the placenta are key to successful prevention of ZIKV infection in developing fetus. To accomplish this goal, they plan to develop a novel heat shock protein (HSP) based vaccine vector that will induce ZIKV-specific responses in the placenta, which should lead to clearance of the ZIKV and prevention of the fetal ZIKV transmission. In the first month of this study, they have started working on cloning the Food and Drug Administration (FDA) approved deoxyribonucleic acid (DNA) vector B45. The B45 vector has in addition to the neomycin and ampicillin resistance genes two expression cassettes, one for expression of HSP glycoprotein 96-immunoglobulin (gp96-Ig) and the other for the ZIKV antigens. They are currently starting to transfect newly cloned B45 plasmid into specific cells to examine the expression of both antigens. Following this aim, they plan to use Western blotting to confirm that they have successfully transfected both

cell lines with the Zika envelope and do not anticipate any set-back in completing their proposed research experiments.
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 #7ZK04:** Point of Care Assay Development for Diagnosis of Zika Viremia

   Principal Investigator: Qun Huo, PhD

   Organization: University of Central Florida

   Abstract of Proposed Research: Conceptual and technological innovations are needed to move beyond the current limits of laboratory-based diagnostic methods used for infectious disease. For emerging infections, such as Zika virus (ZIKV), early detection is particularly important to inform clinical care decisions and to provide real time data on viral transmission and for policy related to vector control. Currently, lateral flow assays to detect anti-ZIKV antibodies cannot detect acute infection and have poor sensitivity. Researchers aim to leverage an existing diagnostic device platform to develop a rapid, point of care (PoC) assay for detecting ZIKV virions and antigens (NS1 and E) in human blood, saliva, and urine during the acute period of infection. In aim 1, they plan to decorate surface acoustic wave (SAW) sensors with anti-ZIKV monoclonal antibodies for nonstructural glycoprotein (NS1) and centromere-associated protein E (E protein), and refine conjugation methods to optimize binding. In aim 2, they will investigate device detection of ZIKV NS1 and E proteins and intact ZIKV in spiked blood, serum, saliva, and urine samples. During this grant period, they successfully developed a nanoparticle assay to detect for acute viral infection. This work is a critical step for developing an accessible, portable, cost-effective PoC device that will facilitate more widespread ZIKV surveillance and early detection. Based on the data from the nanoparticle test, the research team plans to extend their analysis toward a larger panel of clinical samples in the months to come.

   Follow On Funding: None at the time of reporting.

   Collaborations: University of Central Florida (Dr. Karl McKinstry and Dr. Tara Strutt); four students involved in this project are: 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); and University of Miami (Dr. Mario Stevenson); and McGill University (Dr. Martin Richer).

   Journals: Zheng T, Finn C, Parrett CJ, Dhume K, Hwang J, Strutt TM, Sip YYL, McKinstry KK, Huo Q. A nanoparticle enabled blood test for acute viral infection detection. Submitted to ACS Infectious Diseases, April 2017. Revision to be submitted in early August.

   Patents: None at the time of reporting.

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

   Principal Investigator: Karin Y. Chumbimuni-Torres, PhD

Organization: University of Central Florida

Abstract of Proposed Research: The goal of this project is 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, the research team will use a binary probe that has 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 current 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) will exceed the performance of current state-of-the-art approaches for RNA sensing in the following aspects: 1) it will exhibit zero false-positive responses, thus improving sensitivity and limit of detection (LOD); 2) it will enable accurate recognition of SNS at ambient temperatures in RNA; and 3) it will allow for detection of multiple analytes using a single universal probe that is reusable. Therefore, the proposed work plan is to: 1) identify conservative sequences of a variety of Zika strains, 2) enhance the number of specific strains using nucleic acid sequence based amplification (NASBA) and 3) perform the detection using a developed universal electrochemical sensor. Currently, the research team is performing NASBA amplification on a fragment of the ZIKV genome. Optimization experiments are being pursued to validate the sensors and purify the amplified NASBA products.

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 #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

Abstract of Proposed Research: The hypothesis of this study will attempt to prove that ZIKV alters centrosome functions, which then disrupts neural stem cell proliferation or survival, contributing to microcephaly in developing embryos. The researchers' current data shows that the centrosome microtubule-organizing center (MTOC) activity is activated following ZIKV infection and appears to involve Polo-like kinase 1 (PLK1). Based on these data, they will 1) assay changes to centrosome composition and function in ZIKV-infected human neural progenitor cells and other cell lines, 2) test whether PLK1 is activated, and 3) determine whether its PLK1 activation is required for ZIKV to activate centrosomal MTOC activity. In aim 1, they performed pharmacological and genetic engineering experiments to test the requirement of the centrosome and its MTOC activity to support ZIKV propagation. Two centrosome proteins (Ninein and CEP152) have been identified that are responsible for interfering with centrosome function. Although both proteins are linked to genetic syndromes that cause microcephaly, Ninein expressed elevated levels of microtuble-assemby activity at the centrosomes in ZIKV-infected cells. These results show that ZIKV augments centrosomal MTOC activity. The other aims of this project will focus on determining how the interaction between ZIKV protein and host

proteins results in centrosome MTOC activation, and its requirement for ZIKV proliferation. It is also planned to determine whether PLK1 kinase activity and PLK1 activation are required for ZIKV replication and for MTOC activation by ZIKV. By doing so, it is anticipated that the outcome of these experiments will lead to a greater understanding on the mechanisms by which ZIKV impacts centrosome activity to promote its replication, and whether drugs that block these mechanisms can block ZIKV.

Follow On Funding: None at the time of reporting.

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

Journals: None at the time of reporting.

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

   Abstract of Proposed Research: 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 is to develop novel inhibitors of the protease necessary for replication of the ZIKV. To do so, the research team has developed a cell-based reporter system that signals when the protease is active. During the current funding period, they have focused on two aspects of their screening approach. First, they have optimized several assay variables in well plates, and second, they have identified a protease inhibitor (termed ABESF) that could serve as a positive control to help determine the biological end point. By completing this proposed research, the laboratory staff hope to deliver an effective drug screening platform for ZIKV protease activity that will allow for preliminary screening efforts to search for NS2B-NS3 based ZIKV treatments.

   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, Miller School of Medicine

   Abstract of Proposed Research: Prior to the Florida endemic Zika outbreak, Dr. Bandstra's institution was confronted with travel-associated Zika infection in pregnant mothers. Realizing

the magnitude of the situation, the Departments of Obstetrics and Pediatrics developed a Zika Response Team (ZRT), led by Dr. Christine Curry and Dr. Ivan Gonzalez. Their ZRT includes an onsite network of adult and pediatric subspecialties, including Maternal Fetal Medicine and Pediatric subspecialties such as Infectious Disease, Neonatal-Perinatal Medicine, Neurodevelopmental Follow-up, Ophthalmology, Audiology, Neurology, Cardiology, Nephrology, and Neuroradiology. The objective of this proposal is to determine the effect of maternal Zika infection in pregnancy on the acute and long-term outcomes of infants of Zika-positive mothers using serial comprehensive infant growth, physical, neurodevelopmental, ophthalmological, and audiological assessments from birth through age 30 months. Team members in Virology at Florida Gulf Coast University will be pivotal in helping the research team address a related objective to determine the incidence and duration of viral shedding in urine, breastmilk, and saliva, and hopefully whether Zika infection can be transmitted via breastfeeding as documented in other viral infections. The researchers aim to recruit study infants at the University of Miami Miller School of Medicine-affiliated Jackson Memorial-Holtz Children's Hospital and anticipate enrolling 200 infants born to Zika-infected mothers and 100 controls matched (2:1) for sex, race, birth weight and gestational age. Assessments will include comprehensive physical and neurologic examinations, Neonatal Intensive Care Unit (NICU) Network Behavioral Scales, the Bayley Scales of Infant Development-3rd Edition, ocular and fundus examinations, and age-dependent auditory testing.

Follow On Funding: None at the time of reporting.

Collaborations: University of South Florida (Dr. Michael and Dr. Baker)

Journals: None at the time of reporting.

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

   Abstract of Proposed Research: The present application by a consortium formed by the Florida International University and the University of Miami investigators is focused on the entry of 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 bloodbrain barrier (BBB) in this process. They hypothesize that ZIKV infects the brain via underdeveloped or disrupted BBB. This hypothesis will be addressed by infected pregnant mice at different stages of BBB development in embryos. The outcomes of ZIKV infection will be addressed by focusing on neurogenesis of the hippocampal neural progenitor cells and autophagy responses in the brain. Autophagy is a highly conserved biological process responsible for the lysosomal degradation of long-lived proteins, damaged organelles and parts of the cytosol, and has also been implicated in innate and adaptive immune response. Studies have shown that ZIKV exploits autophagy to enhance its replication, and pharmacological inhibitor of autophagosome formation, strongly reduced viral copy numbers in infected fibroblasts. They hypothesize that autophagy plays a key role in the pathogenesis of ZIKV-associated primary microcephaly. Preliminary data supporting this hypothesis show (i) increased expression of the autophagic proteins Beclin1 and LC3, (ii) increased dendritic varicosity in neurons and (iii) increased inflammation with the release of cytokines (IL-6 and

TNF-α) and chemokines (MCP-1 and RANTES) in astrocytes infected with ZIKV. Inducing the autophagy pathway with rapamycin increases ZIKV replication, whereas silencing the autophagy with siRNA against the beclin1 gene decreases viral replication in astrocytes. In this proposal, they plan to use a ZIKV infected- autophagy deficient (B6.129X1-Becn1tm1Blev/J) mouse model treated with anti-ifnar antibody to further evaluate the role of autophagy in the underlying mechanism(s) in ZIKV replication and associated neuropathogenesis. State-of-the-art and recently patented nanotechnology tools will be used to deliver nanoparticles loaded with ZIKV specific anti-ifnar, Beclin 1 and AXL receptor antibodies across BBB to treat or prevent ZIKV induced defects. Thus, this multidisciplinary approach between scientists from the University of Miami and Florida International University exploring a) the role of ZIKV infection on the integrity profile of BBB, b) mechanisms of ZIKA induced pathogenesis and c) nanodelivery of ZIKA specific therapeutic cargo across BBB is uniquely suited to Dynamic Change Team Science.

Follow On Funding: None at the time of reporting.

Collaborations: University of Miami (Dr. Michal Toborek)

Journals: None at the time of reporting.

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

Abstract of Proposed Research: 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 is 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. Work has started, fabricating and testing of interdigitated electrodes (IDEs) on cellulose paper substrate. Also, testing has started on various lysis buffers to find out which buffers have highest impedance, hence will be most suitable for our application.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

    Principal Investigator: Sapna Deo, PhD

    Organization: University of Miami

    Abstract of Proposed Research: Direct detection of Zika virus 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, they are developing a portable, rapid, equipment-free detection technique employing rolling circle amplification of ZIKV RNA followed by paper-strip-based visual detection of the amplified product. Their preliminary data demonstrate 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. 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 get the result. To accomplish their goal, they have formulated the following two specific Aims. Specific Aim 1: Design, development, and analytical optimization of an easy to use, rapid, and cost-effective portable test for Zika virus detection. Specific Aim 2: Evaluation of the test to detect Zika virus spiked in buffer, serum, saliva, and urine. Stability analysis of the developed test. Validation of the test via analysis of clinical samples. The proposed work should result in a rapid, reliable, portable, and cost-effective Zika virus detection test that will facilitate Zika virus detection in developed and developing countries for use in hospitals, community health clinics, and remote locations where the Zika virus is spread.

    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

Abstract of Proposed Research: Transitioning from Africa to the Americas via the South Pacific, Zika virus (ZIKV) infections have become an emerging health pandemic of significant medical importance. Recently, concern about ZIKV infections has increased as the virus has become 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 establishes 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. In severe cases, some Zika patients have required life support. The spread of ZIKV has 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 promote the survival of the ZIKV-carrying mosquitoes and accelerate the spread of the virus. Florida is just behind New York with the highest number of travel-associated cases; the Sunshine state is also one of two states with recorded locally-acquired ZIKV infections (n=139 cases to date). 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. This challenge is attributed to limitations of the current technologies to provide a comprehensive profile of protective neutralizing antibodies against ZIKV infection. In this application, as a team of experts with multiple disciplines in the field of immunology, virology, and public health in the state of Florida, they propose to use 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. The antigen specific products will be isolated and expanded via cloning and recombinant DNA technologies. This extremely efficient process uses much smaller samples than conventional methodologies, and, most importantly, it allows for the identification of even rare cross-neutralizing epitopes. They propose using SCAN technology to identify and isolate ZIKV-specific antibodies that will be evaluated for viral neutralizing properties. Antibodies with neutralizing properties will then be characterized to determine their mechanisms of neutralization. Results will generate a complete repertoire of ZIKV-specific antibodies. While these antibodies will be screened for neutralizing capacity and mapped for antigenic epitope reactivity, on a more fundamental level, they expect their results to establish a simple approach for creating immune- therapeutics and eventually vaccines.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Alexandria Voigt, Semenova T, Janet Yamamoto, Veronique Etienne, and Cuong Q. Nguyen. Therapeutic antibody discovery in infectious diseases using single-cell. Book entitled "Single Cell Biomedicine". Springer-Nature Publishing. Submitted.

Bikash Sahay, Cuong Q. Nguyen, and Janet K. Yamamoto. Conserved HIV epitopes for HIV-1 vaccine. Journal of Clinical and Cellular Immunology. In review.

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

    Abstract of Proposed Research Cell type-based profiling of AXL receptor 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. The project will test the modulation of these pathways in a variety of AXL expressing cell types, as they are differentiated in vitro, because the mechanisms contributing to antiviral responses can vary between cell types as well as across developmental stages. Critical populations of potentially affected vascular, neuronal, glial, retinal, and immune cell types will be used, and the expression of differentiation and viral response-related protein markers specific for each respective lineage will be analyzed. Detailed comparative analysis of gene expression and high-throughput proteomic changes specific to immune signaling will be assessed upon virus exposure. The NSU Cell Therapies Institute will make use of a library of stem cells and chemically defined differentiation platforms coupled with techniques for modulating intracellular viral defense mechanisms that have been developed in collaboration with Karolinska Institute. Altogether this project will establish a strong preclinical rationale for the development of new treatments for improving ZIKV clearance as well as improve their understanding of how ZIKV may contribute to morbidities in systems beyond the fetal brain.

    Follow On Funding: None at the time of reporting.

    Collaborations: Karolinska Institutet, Sweden (Outi Hovatta)

    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

    Abstract of Proposed Research: The brain development of children who were prenatally exposed to ZIKV i.e., Zika-exposed group (Zika PCR and IgM negative and maternal PCR/IgM

positive) is not yet known. Therefore, the aim of this proposal is to characterize longitudinal changes in the brain of children infected with or exposed to Zika virus using advanced brain imaging techniques, and correlate these changes to their neurodevelopmental outcomes. The Zika-PCR positive (n= 15; age range: 0-3 years) and exposed (n=45; age range: 0-3 years) groups will be recruited from the Zika Clinic of University of Miami, Miami. A matching control group (n=15; age range: 0-3 years) will be recruited for comparisons. All subjects will be scanned three times, i.e., at 1-month, 12-month, and 24-30 months of their age. The MRI scans will be performed using a 3 Tesla MRI scanner. The MRI protocol will include conventional diagnostic imaging methods and advanced imaging methods such as MR spectroscopy (MRS), diffusion kurtosis imaging (DKI) and high-resolution tissue structural imaging. Neurodevelopmental outcomes will be assessed at each MRI scan visit and the imaging measures will be associated with the outcomes to evaluate associations between these measures. The metabolites of interest include N-acetyl aspartate, creatine, choline, myo-inositol, glutamate, and lactate. DKI permits us to evaluate alterations in tissue micro-structures, and the metrics obtained include fractional anisotropy, mean diffusivity, axial diffusivity, radial diffusivity, mean kurtosis, axial kurtosis, and radial kurtosis. Volume of calcium deposits will be quantified from diagnostic imaging data. Of great interest is to evaluate the brains of the Zika-exposed children i.e., Zika PCR negative children, to ascertain whether they are normally growing based on their conventional imaging and neurodevelopmental outcomes. Any subtle brain abnormality that is missed by conventional diagnostic imaging will be assessed by more sensitive MRS and DKI techniques. The findings will establish baseline and longitudinal brain imaging abnormalities in Zika-positive and exposed groups of children. Furthermore, the resulting data will allow us to explore associations between their brain imaging metrics and neurodevelopmental outcomes. Preliminary assessment of the MRI study images demonstrates marked atrophy of the brain parenchyma with marked prominence of the extra-axial spaces and severe hydrocephalus. There is lack of normal sulcation in the brain with a smooth gyral pattern noted bilaterally. Multiple tiny punctate foci of susceptibility are noted in the brain parenchyma in keeping with calcifications. These were predominantly localized to the gray-white matter junction bilaterally. Marked volume loss of the gray and white matter was noted in the supratentorial brain parenchyma. MR spectroscopy and diffusion tensor data analysis will be performed after obtaining data from a few more patients.

Follow On Funding: None at the time of reporting.

Collaborations: Jackson Memorial Hospital (three residents/fellows); a Neuroradiology fellow; a third-year postgraduate student; and a fifth-year postgraduate student.

Journals: None at the time of reporting.

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 Alto, PhD

Organization: University of Florida

Abstract of Proposed Research: The Florida Medical Entomology Laboratory (FMEL) is a resource for mosquito entomology. The FMEL is one of the world's largest research institutions devoted to improving the understanding and control of medically important insects. The FMEL

has a repository of mosquito strains and a variety of common and exotic arboviruses available for use in this project. With Firebird's chemistry being developed to detect pathogens in low resource environments, the researchers seek to incorporate Firebird's chemistry into their Zika study. Firebird's chemistry will be adapted to detect Zika virus at points of sampling in either mosquitoes (for public health surveillance) or human samples (urine, for example). This will be possible by the collaboration between Firebird scientists and FMEL scientists. A detection device has been constructed that uses a modified form of Reverse Transcription Loop Mediated Isothermal Amplification (RT-LAMP) in a single tube that serves both to receive the sample as well as, after 30 minutes, to call the presence/absence of an infection. Firebird Biomolecular Sciences has improved on the design of the detection device from an initial prototype. A distributable prototype is now suitable for use by public health services in Florida for demonstration and testing. The University of Florida Principal Investigator has performed infection studies with mosquitoes with Zika virus and tested high temperature and ammonia-detergent mixtures as methods for their universal sterilization.

Follow On Funding: None at the time of reporting.

Collaborations: Undergraduate student from Indian River State college has assisted in tests. He is also an employee of University of Florida, Florida Medical Entomology Laboratory.

Journals: None at the time of reporting.

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 Meckes, PhD

Organization: Florida State University

Abstract of Proposed Research: Recent data suggests that exosomes from the fetus are present in the mother's circulation and may represent a new means to non-invasively monitor the health and development of the fetus. Zika virus is an emerging infectious disease that is rapidly spreading across the Caribbean and South America with confirmed cases of locally-acquired Zika in the state of Florida. 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 as they grow older. Currently, there is no non-invasive test available to determine whether a fetus has been infected with Zika virus or will develop associated disease. This study will take advantage of novel approaches to detect fetal Zika infection and to monitor the health and development of the growing fetus. The objectives for the study are twofold: 1) to compare and characterize fetal-derived exosomes present in blood of healthy and Zika infected pregnant women; and 2) to compare molecular information in exosomes to fetal imaging data acquired during pregnancy. The researchers are well positioned to make significant advances on this area of research as the group has already developed new methods for the isolation and characterization of exosomes from blood, including brain exosomes. Overall, the proposed research will provide a novel way to detect microcephaly risk due to Zika infection by isolating fetal-specific exosomes for early characterization from pregnant women. Methods were established for isolating exosomes from human plasma for molecular characterization by combining size exclusion chromatography with

ultrafiltration. This method was determined to yield exosomes with the highest purity and greatest recovery when compared to other methods. The researchers also created a workflow to sequence messenger ribonucleic acids (mRNAs) and micro ribonucleic acids (miRNAs) from exosomes harvested from cell culture. The researchers now aim to translate these findings to plasma-derived exosomes from patient samples. Initial characterization of exosomes released from Zika infected cells determined the presence of Zika proteins in exosome-containing fractions. The researchers continued efforts to recruit pregnant patients during the reporting period through the collaborators in Puerto Rico. If the researchers can complete the patient recruitment over the next quarter, then there still should be sufficient time to purify exosomes from the plasma and determine the protein and RNA content of the vesicles before the funding period ends. The researchers anticipate that the progress made on isolating and characterizing exosomes from Zika infected cells in the laboratory will help focus and accelerate efforts with patient samples.

Follow On Funding: None at the time of reporting.

Collaborations: Universidad Central Del Caribe.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

    Principal Investigator: Griffith Parks, PhD

    Organization: University of Central Florida

    Abstract of Proposed Research: This project will form a partnership between the University of Central Florida College of Medicine in Orlando and the Moffitt Research Institute in Tampa. 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. The researchers have a long history of studying Complement activation and inhibition by a number of human pathogenic viruses. Thus, the researchers are well positioned to apply their expertise to attack the unique problems in Zika virus biology and immunity. In the ongoing laboratory studies with Zika Virus, the researchers have found that the virus is resistant to Complement-mediated neutralization, a result that is in strong contrast with other related viruses the researchers have worked with before. Most importantly, this resistance to neutralization depends on which particular human donor serum the researchers test. Thus, the researchers 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, these results suggest that there may be human factors that differ between individuals that can control whether the researchers inactivate Zika virus or whether it spread in the body. The long-term goal of their work is to elucidate the interactions of Zika virus with human innate immune systems and how the virus inhibits these pathways to survive and spread through the host. The

short term aims of this project are to build on their new findings to: 1) define the activation and inhibition of human complement pathways by Zika virus, 2) to partner with researchers at Moffitt Research Institute to define the human serum proteins that are associated with Zika virus using cutting edge proteomics technology, and 3) test the hypothesis that Zika virus inhibits Complement-mediated lysis of infected cells. Their work has strong implications for design of novel therapeutics or repurposing of existing drugs that harness the power of Complement to control or prevent Zika infections. Thus far, the progress of this project has included: initiating growth of Zika strain MR766 in insect cells, flasks of mosquito cells were infected at low multiplicity of infection and media was harvested for purification of virus by sucrose gradient, assembled the mosquito research team for a “kickoff' meeting, traveled to Tampa FL at Moffitt Research Center for a "kickoff1 meeting of the Liquid Chromatography – Mass Spectrometry (LC-MS/MS) protein identification team, and grew two species of mosquitoes from eggs.

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.

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

Principal Investigator: John G. Morris, MD, MPH, TM

Organization: University of Florida

Abstract of Proposed Research: To address the challenges of diagnosing Zika in a rapid and cost-effective manner, the researchers propose 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 vRNA detection occurs using blood spotted onto high-quality filter paper instead of FTA cards. Moreover, by using whole blood instead of serum or plasma spotted onto paper, a higher concentration of viruses are deposited and preserved on the filter paper. Finally, RT-SIBA affords rapid and low-cost detection, with results obtainable in around 20 minutes after the vRNA is extruded from dried blood spots (standard real-time RT-PCR requires perhaps 2 hrs). During RT-SIBA, ZIKV vRNA is first reverse-transcribed to complementary deoxyribonucleic acids(cDNA), followed by amplification and detection under low and constant temperature conditions. SIBA relies on recombinase-coated single-stranded invasion oligonucleotides that separate complementary target duplexes that then act as templates for amplification by a DNA polymerase. End-point reads can be performed in real-time polymerase chain reaction (PCR) machines or on a commercially available battery-operated portable fluorescence detection system. Potentially, then, the proposed system is relatively low-cost, rapid, and may lead to Clinical Laboratory Improvement Amendments (CLIA)-waived tests in the future. The Aims of this project include: 1. Validate filter paper as an appropriate medium for the preparation, stability, and shipping of dried blood spots containing ZIKV. Human blood will be spiked with different amounts of ZIKV and spotted onto FTA cards and filter paper, the filters dried, and vRNA extracted and measured by RT-PCR.2. Design and validate an RT-SIBA method for

ZIKV. 3. Integrate the two methods. The combined assay is expected to increase accuracy and reduce false positives/negatives. The researchers have reported successful implementation of an RT-LAMP detection system for ZIKV genomic RNA. Various attempts were made to test the efficacy of the reverse transcription- Loop-mediated isothermal amplification (RT-LAMP) detection system for ZIKV simulated blood samples, then in simulated dried blood samples.

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 Solutions for Florida

    Principal Investigator: Thomas Casale, MD

    Organization: University of South Florida

    Abstract of Proposed Research: As mosquitoes that transmit Zika Virus (ZIKV) are widespread throughout much of the USA and especially Florida, the ZIKV presents 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 will be to develop such 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. An integrated ZiCRN is important for the development of infrastructure necessary to support ZIKV studies that are more complex and require more targeted patient populations. Integrating several sites into a ZiCRN will give investigators access to greater resources including expanded patient databases, diagnostic shared equipment and other resources individual investigators would not otherwise have available. Ultimately, this will aid in attracting research studies and new investigators by having an established infrastructure, and other necessary tools to conduct both inpatient and outpatient clinical research studies. The basic science laboratories that are proposed as part of the ZiCRN can aid in the development of assays to examine predictors of responses to treatments studied and prognosis of patients. The benefits of a ZiCRN to the state of Florida and USF include the potential innovative contributions to medical science, opportunities to provide cutting-edge therapy to the community, enhancement of the image of USF and the state to tackle public health concerns such as ZIKV and improvement of opportunities for USF and the state of Florida to engage in research aimed at counteracting infectious diseases, especially ZIKV. The research team/steering committee hosted a site visit

by Novavax on March 10, 2017. Novavax is developing a new vaccine for Zika and USF is still under consideration as the main site to conduct initial studies. The research team/steering committee hosted a site visit by Mark J. Mulligan, MD, FIDSA, Distinguished Professor of Medicine, Division of Infectious Diseases, Executive Director, The Hope Clinic of the Emory Vaccine Center on June 26, 2017. The researchers are under consideration to receive a subcontract from National Institutes of Health via Emory University for a study entitled “Rapid Research Response to Zika Virus Infections: Humoral and Cellular Responses After Infection in US Residents." The research team has spoken with NIH about conducting phase 2 and phase 3 clinical trials for their Zika vaccine development program. The researchers have filled out a site qualification questionnaire for a phase 3 DNA vaccine trial.

Follow On Funding: None at the time of reporting.

Collaborations: Research team members have solidified the identification of key faculty at USF Health Panama to address new educational initiatives on emerging infectious diseases including Zika. Their team has had several conference calls with the group in Panama and several of us plan to go to Panama this summer to begin education on appropriate conduct of clinical research for Zika. Research team members have contacted USF Medicine International about coordinating/collaborating with the well-established USF Health Panama and USF Health Ecuador partners on vaccine clinical trials. Team members had a conference call with Dr. Nestor Sosa, director of the Gorgas Memorial Research Institute for Health Studies (a USF Health International partner), for discussions on Zika vaccine development, and the possibility of conducting a clinical trial on an acute treatment for Zika.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

    Principal Investigator: Ramzi Younis, MD

    Organization: University of Miami

    Abstract of Proposed Research: In the great state of Florida, the researchers will likely see a prolonged effect of the current Zika epidemic and future outbreaks. Although Zika virus causes relatively mild and nonspecific disease in adults, it has been reported to result in devastating birth defects. At their institution, the researchers have already seen the delivery of approximately 30 infants with exposure to Zika virus infection at birth. A great concern is whether subtle neurologic deficit, such as swallowing, hearing, and vision problems, will affect the long-term development of Zika-exposed infants. The researchers will need to care for the children affected by Zika infection for many years to come. This great responsibility demands that the researchers pursue immediate investigation. Although microcephaly is the better-known complication, reports of swallowing dysfunction, hearing deficits, and eye diseases have been alarming among infants with congenital Zika infection. In addition, Zika-exposed infants born with normal head circumference have been shown to exhibit slowed head circumference growth after birth, implicating a lasting effect of the virus on neurodevelopment. Subtle but progressive feeding difficulty, hearing loss, or vision impairment can be underdiagnosed or missed in infants, with grave consequences for the developing child. The Center for Disease Control and Prevention recognized the threat of craniofacial disorders in Zika infants, and has recommended

additional swallow, hearing, and eye evaluation for infants born with Zika infection. However, health care providers and family members alike are faced with rapidly changing guidelines, unanticipated medical needs of this new disease, and a lack of coordinated plans from the medical professionals. The researchers propose a comprehensive evaluation program with the goal of achieving early diagnosis and intervention for craniofacial disorders in all infants with congenital Zika infection. Drawing from their experience treating children with other congenital defects, the researchers have developed a clear plan to study the characteristics and progression of craniofacial disorders in congenital Zika infection. The inclusion of both symptomatic and asymptomatic Zika infants will allow identification of risk factors for craniofacial disorders, and improve efficiency in the current clinical guidelines. A rigorous evaluation and follow-up schedule by a dedicated team will ensure that signs of craniofacial disorders in all infants affected by Zika infection will be screened appropriately and addressed immediately. The close collaboration of pediatric otolaryngologists, neurologists, speech-language pathologists, audiologists, and ophthalmologists will provide the best care possible for the early development of communicative skills. This program also provides critical educational resources and logistic support for the family and caregivers by facilitating navigation of a complex schedule of medical appointments and triaging new symptoms and concerns. Infants and families will benefit from a coordinated evaluation and treatment plan with a wide array of established health care services to help ameliorate long-term developmental impact.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Mittal, R., Fifer, R.C., Liu X.Z., MD. A Possible Association Between Hearing Loss and Zika Virus Infections. JAMA Otolaryngol Head Neck Surg. Published Oct. 2017. doi:10.1001/jamaoto.2017.1798

Patents: None at the time of reporting.

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

Principal Investigator: Glen Barber, PhD

Organization: University of Miami

Abstract of Proposed Research: 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 has now been detected in South Florida with numerous documented cases of infection occurring in the Miami region. The possibility that ZIKV could become an epidemic worldwide led the World Health Organization to declare ZIKV a global public health emergency. ZIKV was first isolated in the Zika forest of Uganda in 1947. The virus is related to dengue virus (DENV), yellow fever virus (YFV), Japanese encephalitis virus (JAV) and West Nile virus (WNV). The Aedes genus of mosquito is the major vector for ZIKV and has been isolated as far away as Malaysia, as well as Africa and South America. Aside from being transmittable by mosquito, however, ZIKV has now been documented as being sexually transmittable. It is presently unclear whether the United States or regions outside of Brazil will experience a microcephaly endemic. There are presently no therapies or vaccines to treat or

prevent ZIKV infection, respectively, and thus the development of such measures are naturally of paramount importance. Here, the researchers report that the researchers have developed novel, effective vaccines that may protect against ZIKV infection. The researchers intend to further test the effectiveness of their vaccines with the objective of generating sufficient data to warrant the consideration of clinical trials. The researchers are currently preparing all vaccines proposed in the study. These vaccines will be utilized in the proposed Non-Human Primate (NHP) Model at the University of Washington Seattle.

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. Cutting Edge: Innate Immune Augmenting Vesicular Stomatitis Virus (VSV) expressing ZIKA Virus Proteins Confers Protective Immunity. J Immunol. 2017 Apr 15; 198 (8) 3023-3028. doi:10.4049/jimmunol.1602180

Patents: VSV-ZIKA Virus Vector for Treating Zika Virus Infection US Provisional Patent Application No. 62/331,584

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

Principal Investigator: Hugh Fan, PhD

Organization: University of Florida

Abstract of Proposed Research: ZIKV is a major public health concern primarily because it has been linked to abnormally small heads and brains in newborns, a rare condition known as microcephaly. Since ZIKV-infected individuals have common symptoms such as fever, joint pains, and a rash that also occur in other arbovirus infections, ZIKV infection can be misdiagnosed as diseases associated with dengue and chikungunya. It is important to have a point-of-care testing platform to accurately identify ZIKV infection for clinical management of patients. In addition, ZIKV could cause asymptomatic infections. A point-of-service testing platform in the field can be useful for screening asymptomatic patients and monitoring possible ZIKV transmission, as well as for safeguarding the blood supply through ZIKV testing at the blood-donation stations. To address the challenge, the researchers propose to develop laminated paper-based analytical devices (LPAD) for detecting ZIKV infection. LPAD will be developed by borrowing the concept from pH papers and pregnancy test strips. Either colorimetric reading or optical detection can be used. The LPAD devices are of low-cost, easy to operate by nontechnical personnel, and manufacturable. Moreover, their goal is to develop an integrated platform that tests ZIKV infection based on both nucleic acid amplification and immunoassay. To achieve the goal, the researchers propose the following specific aims:1. Design, fabricate, and test one LPAD for ZIKV infection based on nucleic acid amplification. The LPAD device consists of components for lysis, RT-LAMP (loop mediated isothermal amplification), and detection. 2. Design, fabricate, and test another LPAD for ZIKV infection based on IgM antibody. The LPAD device consists of components for antibody conjugation, control/test bands, and colorimetric detection. 3. Integrate two LAPD devices into one platform for multiplexed detection based on immunoassay and nucleic acid amplification. The combined assay is expected to increase accuracy and reduce false positives/negatives. The key advantages of the proposed platform include (1) being portable and low-cost, (2) integration of

genetic test with immunoassay, and (3) ability to screen asymptomatic infections using non-invasive samples such as urine and saliva over a broader testing window. The researchers redesigned the proposed device for ZIKA virus (ZIKV) detection according to their preliminary results and recent tests. Rather than using screw/elastomer microvalves, the researchers have incorporated ball-based microvalves into their device. The part of the device for sample preparation has been fabricated using 3D-printing. It will be integrated with a laminated paper-based analytical device (LPAD). Their platform has been developed to perform ribonucleic acid (RNA) isolation and amplification after ZIKV lysis. The researchers have studied using reverse transcription loop-mediated isothermal amplification (RT-LAMP) to amplify RNA from ZIKV. Their initial results indicate that the researchers can detect 0.1 Pyrococcus furiosus (PFU) ZIKV per device. The researchers have also evaluated two colorimetric detection methods, SYBR Green dye (green stain termed SYBR) and Leuco crystal violet dye, for visually detecting amplified deoxyribonucleic acid (DNA) without a lab instrument. For Aim 2, the researchers have designed a LPAD for ZIKV detection based on immunoassay. We have procured both gold nanoparticle and quantum dots as a detection scheme for the test-strip-based immunoassay. Initial tests with gold nanoparticles showed proper functionality of the LPAD. The researchers have also cultured ZIKV for testing the platforms.

Follow On Funding: National Science Foundation $382,549

Collaborations: None at the time of reporting.

Journals: In this reporting period, a manuscript on fabricating paper-based microfluidic devices has been accepted for publication by Microsystems and Nanoengineering (Nature Publisher Group), authored by Christopher L. Cassano, Teodor Georgiev, and Z. Hugh Fan, with a title of “Using Airbrushes to Pattern Reagents for Microarrays and Paper-fluidic Devices".

Patents: None at the time of reporting.

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 Lockwood, MD, MHCM

Organization: University of South Florida, Morsani College of Medicine

Abstract of Proposed Research: 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 prevention of 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). The researcher 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. This research will first explore the cellular and molecular site(s) of ZIKV that allow its replication in the decidua, then will identify the molecules responsible for ZIKV attachment and infection of decidual cells, resident leukocytes and/or trophoblasts and finally will test novel agents including neutralizing antibodies and small molecule inhibitors that block this attachment and/or infection. In addition, the research team will test these agents in a novel decidual – placental villi explant co-cultures to identify optimal approaches to the prevention of maternal-fetal ZIKV transmission. This strategy will enable the research team to reduce the perinatal burden of ZIKV infection. To accomplish these aims the researcher has assembled an interdisciplinary team of decidual-placental biologists and infectious disease experts.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

    Principal Investigator: Claudia Martinez, MD

    Organization: University of Miami

    Abstract of Proposed Research: 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 present study will enroll patients (18-50 years of age) within six months of Zika virus testing. The primary objective of the proposed research is to examine whether Zika-seropositive compared with Zika-seronegative patients will manifest greater cardiovascular complications over one year following study entry. This will be accomplished by doing a comprehensive cardiovascular evaluation at study enrollment and one-year later in both groups.

    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.

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

Principal Investigator: Matthew DeGennaro, PhD

Organization: Florida International University

Abstract of Proposed Research: 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 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 is to identify the genes that allow mosquitoes to smell repellents to facilitate the design of new mosquito repellents. The 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. By exposing mosquitoes to the insect repellents picaridin, IR3535, oil of lemon eucalyptus (OLE), PMD, and DEET, the team will find the genes that enable each of these chemicals’ repellency. This proposal uses a new technique developed in mammals and Drosophila, Deorphanization of Receptors based on Expression Alteration of mRNA levels (DREAM), that our unpublished data shows can work in mosquitoes. This will allow the research team to comprehensively determine which Aedes aegypti mosquito olfactory receptors are activated by repellents in vivo by characterizing the reduction of mRNA levels of these receptors. The odor-responsive gene sets the researchers will produce will provide insight into the molecular mechanism of mosquito repellency and a list of molecular targets for repellent design. The research team has begun performing experiments for an analysis with RNA-seq using odors and mosquito repellents. The initial RNA isolations and RNA-seq library preparations are in progress.

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 Gonzalez, MD

Organization: University of Miami Miller School of Medicine

Abstract of Proposed Research: Prior to the onset of the Zika outbreak, the University of Miami faced travel-associated Zika infection in pregnant mothers. The Departments of Obstetrics and Pediatrics developed a Zika Response Team (ZRT), with Dr. Christine Curry and Dr. Gonzalez as lead physicians, respectively. The collaboration was developed with one goal in mind, which was to care for both mother and child. This provided the opportunity to create an infrastructure for what was thought to be inevitable. As part of the ZRT, the researchers have a network of adult and pediatric subspecialties available onsite. Once the local transmission of the virus became a devastating reality, the clinical protocols and infrastructure were set in motion. The Obstetrics department’s faculty provides care for the majority of this population. This will

serve as the potential pool from which to recruit subjects into the study. The seroconversion rate thus far is 5%. Extrapolating this rate, of the 5,000 deliveries, it is expected that a pool of about 250 positive women in any given year of ongoing local transmission. The extent of end organ damage related to Zika infection in utero has not been completely elucidated. Mouse model confirms the presence of Zika virus which correlates to detection of virus in urine samples of symptomatic patients, renal involvement has not been evaluated in infected or exposed infants. Hypopigmented lesion related to Zika have also been described but the long-term effect on the retina development has not been defined. Flavivirus infection can also cause myocarditis, yet no data exist in the evaluation of Zika infected or exposed infants for cardiac dysfunction. Immunological response during maternal Zika infection may reveal risk factors for the development of organ damage. Neonates exposed to an in utero infection with Zika can have subtle end organ damage. Therefore, the research team is proposing to investigate newborns infected and exposed to Zika virus in utero. The proposed study will evaluate these newborns over three years to determine links to end organ damage secondary to Zika. End organ damage will be assessed using a combination of established biomarkers associated with organ function and organ images. These will be compared to age specific normative data. Findings will be correlated to Zika immune response and cytokines profiles. The disciplines involved in this study will include virology, infectious diseases, immunology, ophthalmology, cardiology, nephrology and neurology. The institutions involved will include Anne Bates Leach Eye Hospital, University of Miami Miller School of Medicine, Florida Gulf Coast University and the Department of Health in Miami-Dade County.

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.

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

    Principal Investigator: Mario Stevenson, PhD

    Organization: University of Miami Miller School of Medicine

    Abstract of Proposed Research: It is critical that couples planning pregnancy know their infection status and whether either partner may be harboring active ZIKV infection. To minimize the occurrence of ZIKV infection during pregnancy, it is essential to devise guidelines based on the actual duration of ZIKV persistence as well as assays best designed to reveal persistent ZIKV. The researcher proposes to define the nature of ZIKV persistence by assessing the duration of ZIKV RNA in different body fluids (whole blood, plasma, serum, urine, saliva, semen and female genital secretions) obtained longitudinally from men and women with acute ZIKV infection. Furthermore, the team will exploit those samples to validate a singlestep assay for detection of ZIKV RNA in unprocessed samples. The specific objectives include: Aim 1: To evaluate the nature of ZIKV persistence from the duration of ZIKV RNA in different body fluids. The research team will assemble an acute infection cohort comprising men (n=12) and women (n=12). To ensure rapid enrollment, the team will draw on local and international sites. The local site will be Jackson Memorial Hospital/University of Miami, where the majority of travel-related and locally-acquired ZIKV infections in Florida are encountered. International sites will include

Puerto Rico, Dominican Republic, Colombia and Haiti, and will be done in collaboration with Biocollections Worldwide Inc. Screening will be guided by symptomatology, positive serology and positive RT-PCR in blood. Samples will be collected from enrolled subjects at regular intervals over 15 months. Viral RNA will be purified from body fluids and the presence of ZIKV RNA will be determined RT-PCR. Aim 2: 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, singlestep 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 EUA-designated assay for detection of ZIKV RNA in body fluids in which ZIKV RNA persistence is most protracted. The research team proposes that these studies will define the persistence of ZIKV in body fluids and help establish reliable reproductive health guidelines. In addition, the team will validate a point-of-care assay that will guide pregnancy planning in resource-rich and resource-limited settings.

Follow on Funding: None at the time of reporting.

Collaborations: 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 Miller School of Medicine

Abstract of Proposed Research: 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. The Miller School of Medicine, University of Miami is uniquely positioned for this challenge with its expertise in infectious diseases, nanotechnology, biochemistry, and conducting clinical trials. The overarching goal of this project is to investigate nanotechnology-based formulations of the currently FDA approved drug ivermectin to treat Zika virus infection. The optimized formulations will be evaluated through a fast-track clinical trial. Ivermectin, a broadly used anti-helminthic drug, is a highly potent inhibitor of the yellow fever virus. Recent in vitro studies demonstrated inhibitory effects of ivermectin on Zika infection at a relatively higher dose. The research team proposes to create controlled released nanoparticles of ivermectin with ability to supply slow therapeutic dose of this drug over a prolonged period of time. Nanoparticles will also result in prolonged circulation thus effectively increasing the therapeutic window. The research team has three specific Aims: Aim 1: Optimization of nano-formulations of ivermectin for oral use. Aim 2: In vitro and preclinical evaluations of ivermectin nano-formulations. Aim 3: Develop rapid implementation of the clinical trial network using the currently existing facilities. Perform Phase I Clinical Trials with our newly developed nano-formulations of ivermectin.

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 Monteiro, PhD

    Organization: Moffitt Cancer Center

    Abstract of Proposed Research: The Zika virus (ZIKV) has recently been documented in the state of Florida, raising concerns about its potential long-term health effects on those infected. ZIKV infection has been shown to lead to disruption of neural progenitor development in mice and in human cells. Emerging evidence suggests that ZIKV has the potential to cause Alzheimer's disease style damage to the adult brain as well. 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, the research team hypothesizes 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 will determine the ZIKV protein-Human protein interaction network using a combination of state-of-the art methodologies. The research will focus on ZIKV non-structural proteins and on Brazilian isolate-specific missense variants. Cellular targets of ZIKV proteins will be validated and their biological significance determined in human neural progenitor cells and in a human cerebral organoid model. The research laboratory is well-positioned to rapidly complete this pilot due to the research team's systems biology expertise in protein-protein interaction networks, including prior projects focused on proteins implicated in microcephaly. In addition, they have also implemented and optimized the use of a human cerebral organoid model. The team expects to generate a comprehensive interaction map of ZIKV proteins and their interaction partners in human cells. They will have assessed the extent to which interactions of ZIKV proteins with a cellular protein leads to cell dysregulation and malformations in a human cerebral organoid model. This interaction network will help elucidate the biological effects of ZIKV infections and create a basis for the discovery of drugs that could abrogate or attenuate the brain health effects.

    Follow on Funding: None at the time of reporting.

    Collaborations: Federal Institute of Rio de Janeiro, Rio de Janeiro, Brazil (Dr. Marcelo Carvalho and Dr. Rafael Mesquita)

    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 Brown, PhD

    Organization: University of Florida

Abstract of Proposed Research: There is currently no vaccine or antiviral therapy licensed for the treatment or prevention of Zika Virus (ZIKV). This project aims to address optimizing effective antiviral therapies for ZIKV infections to prevent the spread of the virus via sexual contact. The research team's strategy is to explore the antiviral activity of anti-infective agents that are already approved for clinical use. Drug repurposing is an efficient and cost-effective method to rapidly accelerate drug development. The team will evaluate four different drugs (ribavirin, interferon-α, favipiravir, and ivermectin), all which have demonstrated antiviral potential against ZIKV. The antiviral activity of all four agents will be examined against two clinically relevant ZIKV strains (one of South American Lineage and one of African Lineage) using human cervical and prostate cell lines as in vitro models of genital infection in both males and females. For antiviral therapy to be successful, one must first identify the optimal dose and dosing interval for each compound. The researchers will use a state-of-the-art model system to simulate fluctuating drug concentrations following the administration of a drug due to biological processes such as absorption, metabolism, and excretion. This allows the team to evaluate the impact of different doses and dosing intervals on the antiviral activity of each agent. Based on the observed drug concentrations and viral loads, novel mechanism-based mathematical models will be developed to design regimens that yield the best therapeutic outcomes in man. These rationally optimized regimens will have the greatest likelihood of clinical success, since they maximally inhibit viral replication and suppress resistance. At the completion of this project, the results can be directly translated to the design of future clinical trials.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Camilly P. Pires de Mello, Xun Tao, Tae Hwan Kim, Jürgen Bulitta, Jaime L. Rodriguez, Justin J. Pomveroy, and Asley N. Brown. Zika virus (ZIKV) replication is substantially inhibited by novel favipiravir and interferon-alpha combination regimens. 2017. Under peer-review at *The Journal of Infectious Diseases.*

Patents: None at the time of reporting.

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

Principal Investigator: Mark Sharkey, PhD

Organization: University of Miami Miller School of Medicine

Abstract of Proposed Research: While viremia is short-lived (typically less than one week), it has now become apparent that Zika Virus (ZIKV) may persist for longer periods of time in fluids such as urine and semen (six months or more), and there have been reports of sexual transmission of infection weeks after the initial infection in an index patient. Thus, the ability to diagnose the infection in different body fluids is critical for transmission prevention and for reproductive health decisions. A simple, inexpensive, point-of-care assay that does not require sophisticated equipment or extensive operator experience is needed. The research team has developed an assay based on standard PCR that uses a novel enzyme with both RNA and DNA-dependent polymerase activities and the capability to amplify RNA directly from clinical samples without a purification step or separate cDNA synthesis. The incorporation of a fluorescent probe into the PCR reaction allows immediate scoring of positive reactions with an inexpensive blue light source. This format brings the cost and complexity of ZIKV diagnosis down to a fraction of what they currently are with standard RT-qPCR assays and makes feasible

a cost-effective point-of-care assay. The proposed research will have two specific Aims: Aim 1: Evaluate the assay for sensitivity and specificity in body fluids (i.e. spiked lab samples and clinical samples) including blood, urine, saliva and semen, and Aim 2: Validate a point-of-care kit in clinical samples.

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.

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

Abstract of Proposed Research: Obstetrical ultrasound has been used to evaluate neurological damage in the fetus caused by ZIKV. Although ultrasound can adequately diagnose microcephaly, it has limited sensitivity and specificity. Unfortunately, there are a variety of other neurological disorders that can develop in fetuses exposed to ZIKV infection, most of which are not identifiable by ultrasound. The goal of this project is to utilize fetal MRI with DTI as a screening test in ZIKV infected patients to assess for potential subclinical neurological disorders in the developing fetus. To achieve this goal, a multi-disciplinary team was formed from internationally renowned imaging scientists, radiologist, statistician, and biomedical engineer(s). In the current collaborative effort, their intent is to develop quantitative MRI and DTI analysis platform to explore neurological disorders caused by ZIKV infection. The Principal Investigator had the opportunity to meet world leaders in fetus imaging at the International Precious Metals Institute (IPMI) conference. After talking to experts from MIT, Harvard, University of North Carolina, and Austria, it was concluded that during the limited scanning time of each brain of the fetus should be the focus and less time should be spent for protocols in imaging body composure. New protocols were determined and found to reflect this fact. 3T Philips Achleva scanner equipped with a specifically developed 32-channel neonatal receiver coil integrated with patient handling system will be used for this study. Diffusion MRI will be employed Multiband 4 spin echo EPI with 4 interleaved phase encode directions to acquire 300 volumes arranged on 4 b-shells (20 bOs, and b=400, 1000, 2600s/mm2 sampled in ratios 64:88:128), 1.5x1.5 voxels, 3mm slices with 1.5mm overlap, 3800/90ms TRITE. We continue software development with tissue classification problem in MRI side. Dr. Bagci was invited to the informal biweekly calls of NIH-Zika-initiative both in clinical and pre-clinical discussions of Zika.

Follow On Funding: None at the time of reporting.

Collaborations: University of North Carolina (Dr. Yap); NIH-Zika-initiative; and Oxford University (Dr. Stamatis Sotiropolous).

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

    Abstract of Proposed Research: 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 NAAT, such as 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 is 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. The platform offers highly selective and sensitive, low-cost and instrument-minimized format for detection of natural RNA molecules. The ZIKV defection procedure will include the following stages: (i) brief pre-processing of the biological specimen (blood, serum etc.); (ii) incubation of the analyzed sampled with reagent 'A' at 65oC for 15-30 min; (iii) adding reagent 'B' followed by incubation for 15-30 min at 65oC; (iv) adding reagent 'C', incubation at room temperature for 5-10 min; and (v) registering color change if ZIKV infection is present. The applications of the proposed platform can be extended to other flaviviruses in the frame of follow up studies. Three fragments of ZIKV-genome were selected as targets for vsDz probes - two in the gene encoding Envelope (E) protein and another in the gene encoding NS5 protein.

    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 Teng, PhD

    Organization: University of South Florida

    Abstract of Proposed Research: 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 proposes 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. Further, they propose to use an innovative approach to the discovery of natural products as drugs and/or scaffolds for subsequent medicinal chemistry development. Discovery of lead compounds from their screen will allow development of ZIKV-specific drugs and identify scaffolds for further medicinal chemistry development.

A young woman presented in labor to the UM-affiliated Jackson Memorial-Holtz Children's Hospital and delivered a full-term female infant who was highly suspected of having Congenital Zika Syndrome (CZS) due to severe microcephaly. This first enrollee is the subject of an important case report for submission to the New England Journal of Medicine. The mother lived in a neighborhood contiguous to one of the four CDC-declared Zika outbreak zones in Miami. The infant was conceived last summer during the height of the outbreak. A 20-week fetal ultrasound did not detect anomalies, but the 36-week fetal ultrasound showed poor fetal growth, microcephaly, ventricular dilatation, and suspected agenesis of the corpus callosum. The infant's physical exam at birth showed intrauterine growth restriction, microcephaly, and hypertonicity of the extremities.

Follow On Funding: None at the time of reporting.

Collaborations: University of South Florida (Michael Teng and Bill Baker); and University of Miami Miller School of Medicine (Ms. Samantha Langer, second year medical student).

University of Washington in St. Louis (Ms. Kristen Smalling)

Journals: None at the time of reporting.

Patents: None at the time of reporting.