Biomedical Research Advisory Council

William G. “Bill” Bankhead Jr., and David Coley Cancer Research Program

James and Esther King Biomedical Research Program

Live Like Bella Pediatric Cancer Research Initiative

2020-2021 Annual Report

Ron DeSantis
Governor

Joseph A. Ladapo, MD, PhD
State Surgeon General

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BIOMEDICAL RESEARCH PROGRAM INTRODUCTION AND OVERVIEW

Since 2001, the Florida Legislature has recognized the need to support vital research conducted in both academic and private institutions throughout the state through the William G. “Bill” Bankhead Jr. and David Coley Cancer Research (Bankhead-Coley) Program (section 381.922, Florida Statutes) and the James and Esther King Biomedical Research (King) Program (section 215.5602, Florida Statutes). The Live Like Bella Pediatric Cancer Research Initiative (Bella) is now in its fifth year of funding and is the only state-funded pediatric cancer research program in the country. Total funding, in the amount of $18,505,007, was awarded to Bankhead-Coley, King, and Bella grantees. This funding during Fiscal Year (FY) 2020-2021, resulted in 13 Bankhead-Coley, 12 King, and 11 Bella new research grants. These awards are made to universities and cancer research centers across the state, to support researchers for improving prevention, diagnosis, and treatment.

Research grants are awarded through a competitive peer review process. Awards are based on scientific merit, as determined by independent peer review by experts located outside Florida who are free from conflicts of interest. Full-time researchers at any Florida-based university or established research institution are eligible to apply. All researchers provide a legislative report that is used to produce in this annual report. Per statutory requirements, the progress report includes the following information:

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

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

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

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

- Recommendations to further the mission of the programs.

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

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

- Attracting new research talent and grant-producing researchers.

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

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

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

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

LIVE LIKE BELLA PEDIATRIC CANCER RESEARCH INITIATIVE

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

- Significantly expand pediatric cancer research capacity in Florida.

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

- Reduce the impact of pediatric cancer on disparate groups.

JAMES AND ESTHER KING BIOMEDICAL RESEARCH PROGRAM

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

- Improve the health of Floridians by researching better prevention, diagnoses, treatments, and cures for cancer, cardiovascular disease, stroke, and pulmonary disease.
- Expand the foundation of biomedical knowledge relating to the prevention, diagnosis, treatment, and cure of diseases related to tobacco use, including cancer, cardiovascular disease, stroke, and pulmonary disease.
- Improve the quality of the state's academic health centers by bringing the advances of biomedical research into the training of physicians and other health care providers.
- Increase the state's per capita funding for research by undertaking new initiatives in public health and biomedical research that will attract additional funding from outside the state.
- Stimulate economic activity in the state in areas related to biomedical research, such as the research and production of pharmaceuticals, biotechnology, and medical devices.

BIOMEDICAL RESEARCH GRANTS RETURN ON INVESTMENT

In 2020, the Florida Department of Health (FDOH) conducted a survey of researchers who received Biomedical Research Grants to determine long-term results. Key findings reported by researchers include:

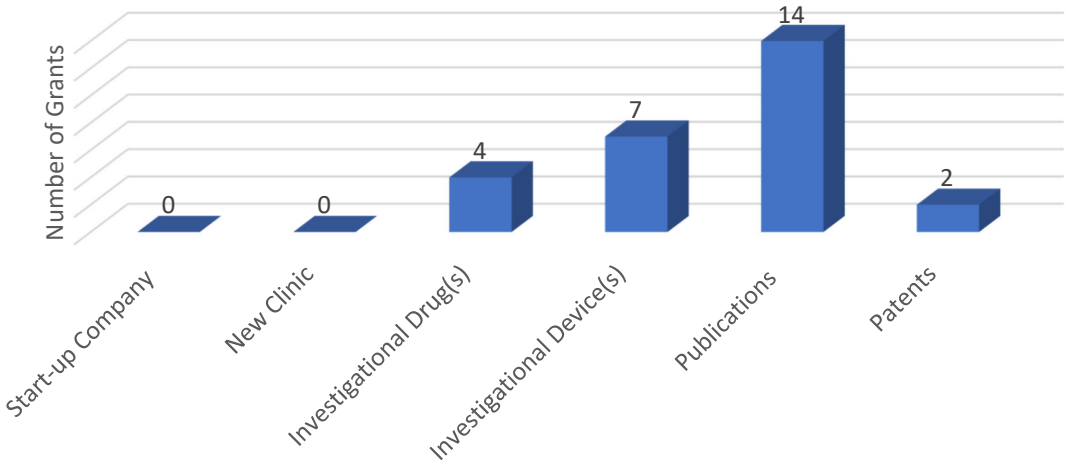
- 58% of research conducted remains active;
- 38% reported that receiving these grants assisted them in receiving tenure;
- 65% of positions created were made permanent;
- 20% reported moving to Florida in anticipation of applying to these funding opportunities; and
- 20% reported recruiting researchers from out of state to work on the grant.

Table 1: Awarded Institutions 2006-2021

All Children's Research Institute	Florida State University	Saneron CCEL Therapeutics
Ava Maria University	Haley VA Hospital	Sanford-Burnham Presby
Bay Pines VA Health Care System	M.D. Anderson Cancer Center	South Florida Veterans Affairs Foundation
Carlos Albizu University	Mayo Clinic	The Scripps Research Institute
Edward Waters College	Miami Cancer Institute Baptist Health South Florida	Torrey Pines Institute
Florida Agricultural and Mechanical University	Moffitt Cancer Center	University of Central Florida
Florida Atlantic University	Nano Discovery, Inc.	University of Florida
Florida Hospital Cancer Institute	Nemours Children's Clinic	University of Miami
Florida Institute of Technology	Nova Southeastern University	University of South Florida
Florida International University	Roskamp Institute	University of West Florida

Self-reported by the researchers that continue to make advancements on the research funded by the state of Florida the chart (Figure 1) below indicates that the researchers had significant success in achieving Food and Drug Administration (FDA) approval for Investigational New Drugs (IND). Intellectual property and patents are also difficult to achieve, but Florida researchers have been successful in achieving these milestones.

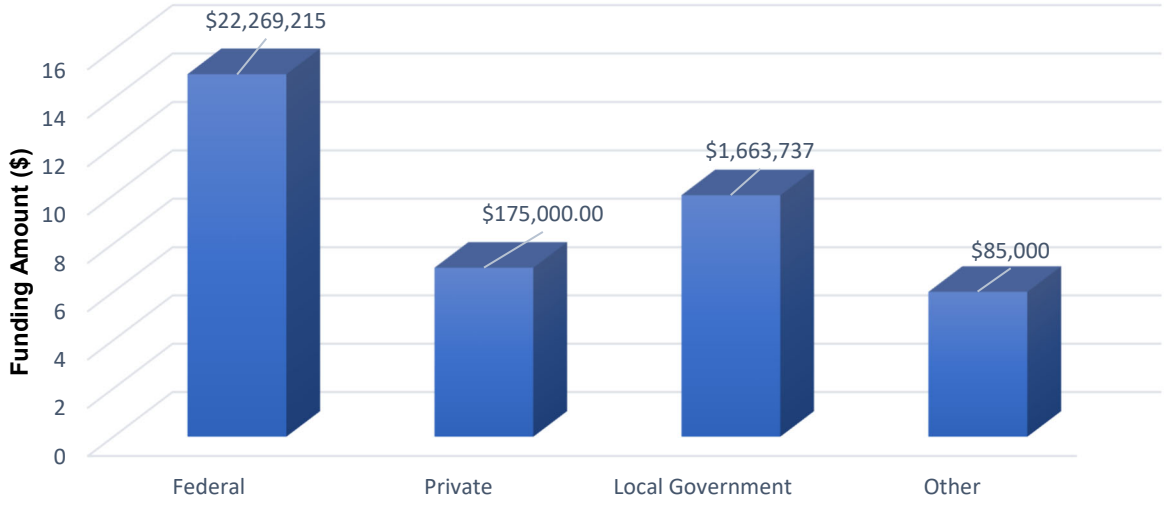
Figure 1: Impact of Completed Research from 2014-2019



Also in Figure 2, the research survey show state research dollars result in researchers qualifying for new funding to continue research. Florida researchers are effective in receiving follow-on funding. Based on survey data, 68% of researchers indicated receiving additional

funding to continue the research. This brings new funding into the state and builds economic stability for research.

Figure 2: Additional Grant Funds Awarded for Florida Research Continuation (Follow-on Funding 2014-2019)



BIOMEDICAL RESEARCH GRANT ADVISORY BOARD OVERVIEW AND MEMBERSHIP

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

(1) Providing advice on program priorities and emphases.
(2) Providing advice on the overall program budget.
(3) Participating in periodic program evaluation.
(4) 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.
(5) Assisting in the development of appropriate linkages to nonacademic entities, such as voluntary organizations, health care delivery institutions, industries, government agencies, and public officials.
(6) Developing criteria and standards for the award of research grants.
(7) Developing guidelines relating to solicitation, review, and award of research grants and fellowships, to ensure an impartial, high-quality peer review system.
(8) Reviewing reports of peer review panels and making recommendations for research grants and fellowships.
(9) Developing and providing oversight regarding mechanisms for the dissemination of research results.
(10) The council shall select, by majority vote, six members of the council who must combine with seven members of the Florida Cancer Control and Research Advisory Council to form a joint committee to develop performance measures, a rating system, a rating standard, and

an application form for the Cancer Center of Excellence Award (section 381.925, Florida Statutes).

The names and positions of each Biomedical Research Grant Advisory Council Member, as of June 2021, are listed below. There are currently two vacancies. (Biographical statements or curriculum vitae available upon request):

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

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

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

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

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

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

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

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

Vacant Seat: American Lung Association

Vacant Seat: House of Representatives

Strategic Goals

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

- Prevention and Treatment

  - Conduct research with a focus on prevention and improved treatment or care delivery that contributes to decreased deaths due to lung cancer by 15%, 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, central nervous system (CNS) tumors, myeloma, leukemia/myelodysplastic syndrome) that result in significant improvement in survival/quality of survival in adults and children in at least two of these cancers.

  - Enhance understanding of the relationship between obesity, healthy weight, and cancer.

  - Improve screening accuracy, detection of high-risk subgroups, and/or improved implementation of cancer screening programs that result in a 20% increase in early detection of cancer or preventable cancer within 10 years.

- Technology Transfer Feasibility (TTF)

  - Establish at least five Investigational New Drug applications or Investigational Device Exemptions based on Florida investigator drug discovery, biologic, or other therapeutics that result in at least two multi-center collaborative clinical trials within 10 years.

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

- Health Disparities

  - Develop research that contributes to reductions in deaths due to lung cancer by 30%, 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).

- IND or IDE
    - Supports the development of IND and IDE applications to the FDA as part of an application for marketing. The intent is to support promising new drug discovery and commercialization of new drugs.

Funding cycle year 20-21 awards were made to support the following research priorities for Bankhead-Coley, King, and Bella initiative grants:

23 Awards – Prevention and Treatment: (10 Bankhead-Coley, 6 King, and 7 Bella) These awards focus on research with a focus on prevention and improved treatment or care delivery that contributes to a reduction in deaths in at least one of the following types of cancers: pediatric, lung, breast, prostate, colon, or melanoma.

No Awards – Technology Transfer: The goals of this grant mechanism is 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.

3 Awards – Health Disparities: (1 King, and 2 Bella) This research contributes to reductions in deaths due to the cancers listed above resulting from health disparities due to race, ethnicity, or income.

2 Awards – Tobacco Use: (2 King) This research focuses on reducing tobacco use in children, adolescents, and adults through cessation.

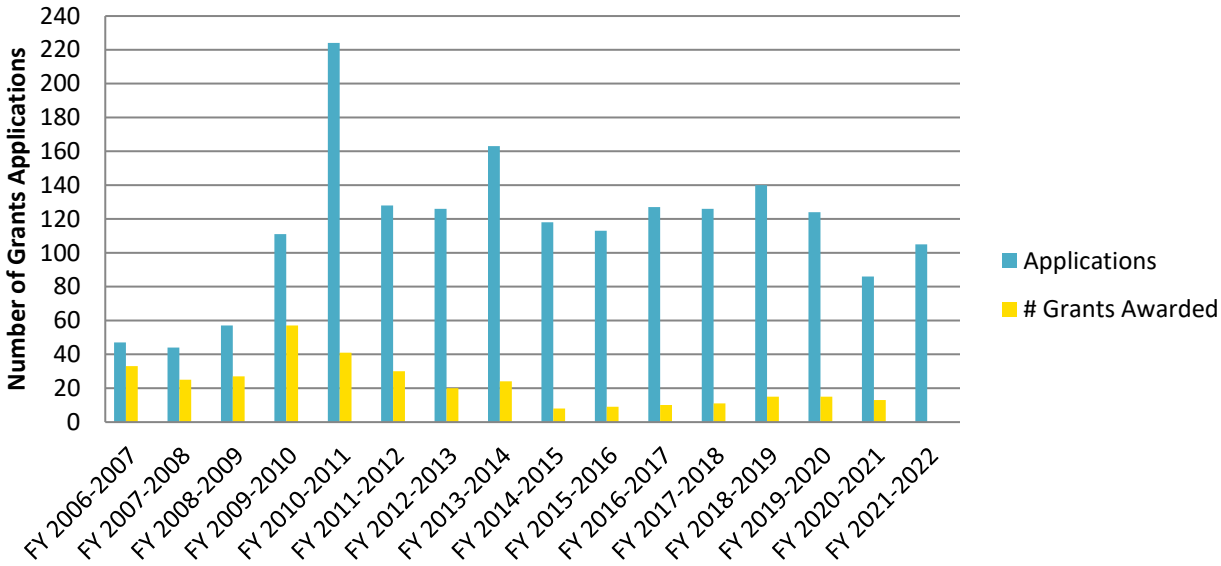
1 Award – Screening: (1 Bella) This research priority focuses on improving screening accuracy, detection of high-risk subgroups, and/or improved implementation of a cancer screening program that results in an increase in early detection or prevention of at least one of the cancers listed above.

4 Awards – Treatment-Related Morbidities: (2 Bankhead-Coley and 2 King) This priority expands upon research that improves scientific understanding of causes and subsequent impact of cancer/cancer-treatment related morbidities in other systems (e.g., cardiovascular, pulmonary, endocrine, lymphatic, CNS, reproductive, developmental impairment, graft-versus-host disease).

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

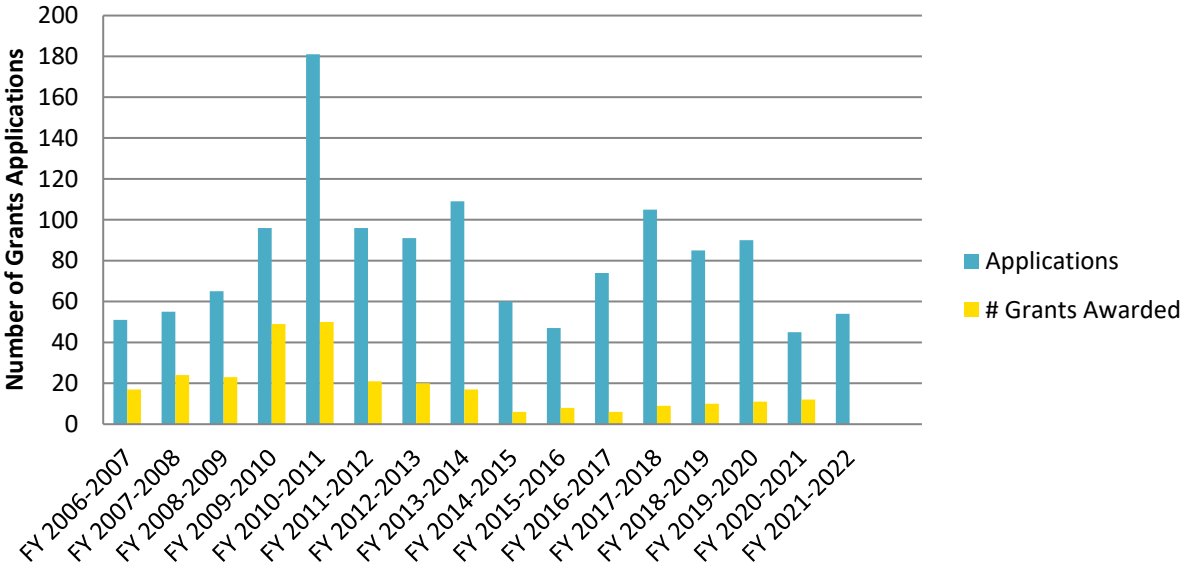
1 Award – The Impact of Obesity: (1 King) This award is investigating the impact of obesity on renal cancer.

Figure 3: Bankhead-Coley Applications and Funded Projects



In FY 2020 - 2021, 86 grant applications were submitted in response to the Bankhead-Coley funding opportunity announcement, and 13 cancer-related disease research projects were awarded.

Figure 4: King Applications and Funded Projects



In F Y 2020 - 2021, 45 grant applications were submitted in response to the King funding opportunity announcements, and 12 tobacco-related disease research projects were awarded.

Figure 5: Bella Applications and Funded Projects

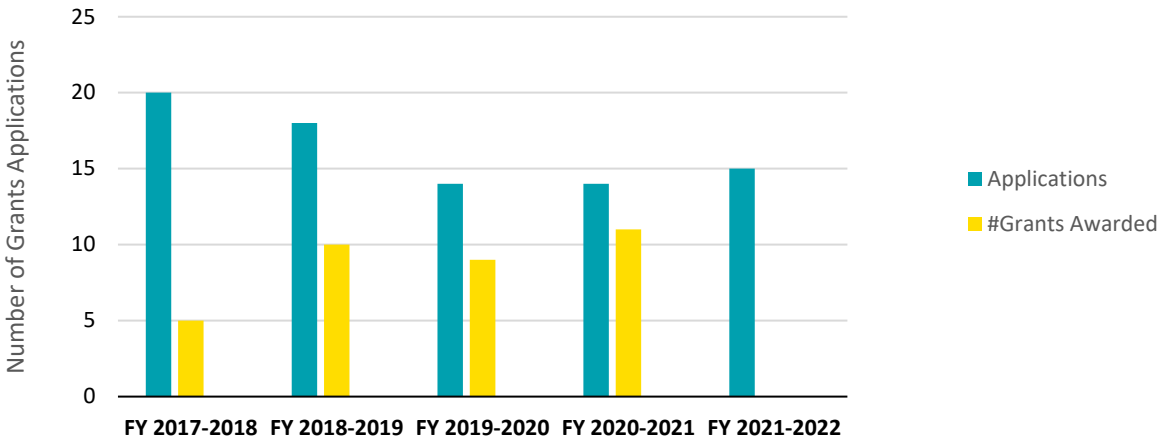


Figure 3: In FY 2020 - 2021, 14 grant applications were submitted in response to the Bella funding opportunity announcement, and 11 pediatric cancer research projects were awarded. As the program continues to become known, it is anticipated that more grant applications will be submitted.

NIH RESEARCH FUNDING AND FLORIDA'S RANKING

For the past five years, the state of Florida has remained 12th in the U.S for the total amount of federal funding awards. There was an increase in the total amount of funding for FY 2020-2021.

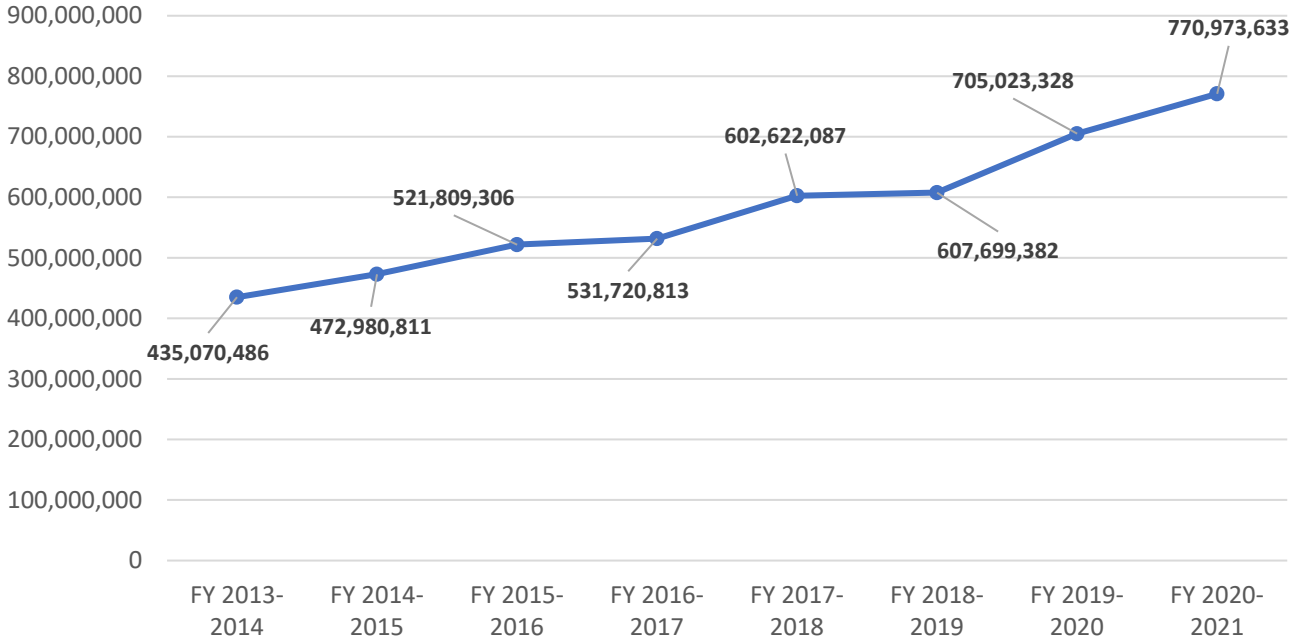
Figure 6: NIH Research Funding from the FY 2020 - 2021 Reporting Period

NIH Biomedical Research State Funding and Rankings FY 2020 - 2021		
State	**Total Funding**	**Rank**
CA	$4,905,031,116	1
NY	$3,543,320,580	2
MA	$3,164,158,511	3
PA	$1,988,775,048	4
NC	$1,873,319,584	5
TX	$1,606,479,921	6
WA	$1,344,742,452	7
MD	$1,262,411,317	8
IL	$1,054,486,219	9
OH	$892,848,570	10
MI	$865,958,839	11
FL	$770,973,633	12
MO	$746,454,293	13
GA	$702,392,789	14
CT	$664,152,578	15
TN	$642,869,598	16
MN	$623,985,338	17
WI	$533,561,704	18
CO	$502,234,964	19
VA	$447,622,633	20

The top 20 states receiving NIH funding are displayed. With over $770 million in NIH funding, Florida is ranked 12th in the nation. (*Source: NIH Research Portfolio Online Reporting Tools (RePORT)*

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

Figure 7: NIH Funding for Florida FYs 2013 - 2014 through 2020 - 2021



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

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
21B01	H. Lee Moffitt Cancer Center	Kenneth Tsai, MD, PhD	$530,900	4/30/2024	No	No	No
21B02	H. Lee Moffitt Cancer Center	Brian Czerniecki, MD, PhD	$1,327,221	4/30/2026	No	No	No
21B03	University of Miami	Thomas Malek, PhD	$530,880	4/30/2024	No	No	No
21B04	H. Lee Moffitt Cancer Center	Florian Karreth, PhD	$530,880	6/30/2024	No	No	No
21B05	University of Florida	Andrew R. Judge, PhD	$530,880	4/30/2024	No	No	No
21B06	H. Lee Moffitt Cancer Center	Gina DeNicola, PhD	$530,880	4/30/2024	No	No	No
21B07	University of South Florida	Rex Philpot, PhD	$528,130	4/30/2024	No	No	No
21B08	H. Lee Moffitt Cancer Center	Relinquished					
21B09	H. Lee Moffitt Cancer Center	Kathleen Egan, ScD	$1,327,120	4/30/2024	No	No	No
21B10	University of Miami	Noula Shembade, PhD	$530,470	4/30/2024	No	No	No
21B11	Florida State University	Jerome Irianto, PhD	$265,440	4/30/2024	No	No	No
21B12	H. Lee Moffitt Cancer Center	Matthew Schabath, PhD	$1,327,180	4/30/2026	No	No	No
21B13	University of Florida	Zhijian Qian, PhD	$530,880	4/30/2024	No	No	No

1. **Grant #:** 21B01 Sensitizing Melanoma to Immunotherapy

   Principal Investigator: Kenneth Tsai, MD, PhD

   Organization: H. Lee Moffitt Cancer Center

   Abstract: The advent of immunotherapy has revolutionized cancer therapy. Even in the most favorable of circumstances such as in melanoma single agent response rates generally have not exceeded 55% on average. Resistance remains unaddressed and despite considerable effort, effective rationales for combinations of targeted agents and immunotherapies are largely lacking. Approximately 30% of cutaneous melanomas are driven by activating mutations in Neburoblastoma rat sarcoma viral oncogene (NRAS). This subset of melanoma is generally less responsive to immunotherapy and has no available targeted therapy. First, we propose that congenital nevi are an excellent model for testing chemopreventative measures for NRAS-mutant melanomas because they are overwhelmingly caused by NRASmutations, they transform into melanoma at a high rate (10-30%), and are not known to respond to immune (imiquimod) or targeted therapy (MEK inhibitor). Second, we propose that our approach can be generalized to sensitize established NRAS-mutant melanomas to immunotherapy. Approaches to dampen ERK signaling downstream of mutant NRAS have failed to yield meaningful clinical responses in melanoma and congenital nevi. Yet pathway agonism has not been explored. Interestingly, many BRAF inhibitors (BRAFi) paradoxically activate ERK in RAS-mutant cells. It

occurred to us that inducing paradoxical ERK activation in pre-malignant and established RAS-mutant cancers, might elevate ERK signaling enough to trigger oncogene-induced senescence. Our data show that 15 definitively genotyped RAS-mutant cancer cell lines of diverse lineages arrest whenexposed to clinically-relevant doses of BRAFi in culture and in-vivo. The complete lack of response using a BRAFi incapable of paradoxical ERK activation, and the dependence of the arrest on hyperactive ERK, strengthens this argument significantly. Importantly, when employed in an immunocompetent, C57BL/6 mouse model of NRASQ61R-driven melanoma, BRAFi-induced ERK hyperactivation synergizes with anti-PD1 to induce tumor regression, accompanied by peritumoral CD8+ T-cell infiltration and activation and reduction of myeloid suppressor cells. In vitro, BRAFi-induced ERK hyperactivation results in senescence with features of oncogene-induced senescence and the classical senescence-associated secretory phenotype. Our central hypothesis is that ERK hyperactivation will induce senescence and an inflammatory microenvironment that sensitizes NRASmutant nevi and melanomas to topical and systemic immunotherapy, respectively. In Aim 1, we will test the hypothesis that the genetic and pharmacologic induction of ERK hyperactivity induces the expression of inflammatory mediators in NRAS-mutant melanocytes and nevi, and melanomas. From this, we will identify core transcriptional and signaling modules which drive senescence and cytokine secretion. We will characterize the microenvironmental effects of BRAFi in a mouse model of NrasQ61R-driven congenital nevi and melanoma. In Aim 2, we will test the hypothesis that genetic and pharmacologic ERK hyperactivation can sensitize NRAS-mutant nevi to topical Toll-like receptor agonists (imiquimod) and NRAS-mutant melanomas to anti-PD1 immunotherapy, and determine the optimal sequencing of therapies. This proposal addresses the focus areas of prevention and treatment of melanoma and details an approach to bypass primary and acquired resistance to immunotherapy. We advance a clinically tractable strategy to use oncogenic pathway agonism to prevent and treat cancer.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

2. **Grant #:** 21B02 Overcoming Resistance in HER2 Breast Cancer Through a Novel Immunotherapy Approach

Principal Investigator: Brian Czerniecki, MD, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: Despite the utilization of targeted therapies like Herceptin, HER2 invasive breast cancer patients presenting with advanced or metastatic disease often develop resistance to these therapies and eventually die from the disease. We have made two novel observations in human HER2 breast cancer cells and mice that together can overcome resistance in HER2 breast cancer. The first observation is that an immune system protein interferon gamma when combined with targeted therapies even in HER2 resistant cells makes them sensitive to the

induction of tumor senescence so that they can no longer replicate and as a result makes them easy targets for the immune attack. The second observation is that delivery of intratumoral dendritic cells (the cell responsible for starting immune responses) in combination with an antibody that enhances the ability of lymphocytes making interferon gamma better able to enter the tumor, in mouse models completely eradicates metastatic HER2 tumors. This effect is mediated by interferon gamma produced by CD4 Th1 cells, B cells producing HER2 antibodies as well as killer cells of the immune system. In bilateral tumor models, injecting only one tumor with dendritic cells in combination with the antibody enhancing T cell infiltrates makes the other untreated tumor disappear equally well (abscopal effect). Interestingly, ex vivo expansion of CD4+T cells (from tumor regressed immune mice) in the presence of IL-7 cytokine generates CD4 Th1 with stem properties and when adoptively transferred to mice bearing HER2+ tumors significantly improved anti-tumor immune response with 50% tumor regression in HER2+ murine model. This procedure was utilized to expand CD4 T cells from the blood of DC vaccinated Breast Cancer patients that we can grow exponentially out of the body to transfer back with intratumoral dendritic cells to treat even large volume metastatic disease. We are now ready to translate these findings to patients with metastatic HER2 refractory breast cancer. In this proposal we will develop a novel Phase I/II clinical trial in HER2 metastatic refractory patients that first administers autologous HER2 pulsed intratumoral dendritic cells combined with systemic anti-semaphorin 4D to determine safety and response. The research staff will then adoptively transfer their own anti-HER2 CD4 Th1 cells that we expand in IL-7 these stem-like T cells ex vivo from their peripheral blood and administer in combination with autologous dendritic cells and anti-semaphorin 4D to determine safety and response of this combination. We will measure the patient's immune response in peripheral blood, determine antibody production, and measure tumor and immune parameters as a result of therapy as well as measure response in accessible tissues. (3) We will investigate the mechanism by which interferon gamma modulates this effect in HER2 resistant cells. This therapy is innovative and based on solid preclinical work developed from observations in our laboratory. If successful this therapy will reduce the mortality of HER2 breast cancer and will aid in the translation of immunotherapy into early clinical trials in HER2 breast cancer. The clinical protocol is already developed and approved by the Moffitt Scientific Review Committee. These patients are usually refractory to HER2 based therapies and we propose getting Th1 interferon secreting cells into the tumor using this regimen will make these tumors again sensitive to HER2 targeting agents improving survival.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

3. **Grant #:** 21B03 CD4+ T Effector Cells in Cancer Immunotherapy

   Principal Investigator: Thomas Malek, PhD

Organization: University of Miami

Abstract: Immunotherapy is now a frontline treatment for several cancers. The most effective form of immunotherapy is checkpoint blockade, where specific antibodies are used to “block” physiological signals that normally limit an immune response, including anti-tumor immunity. Despite the therapeutic benefit, many cancer patients show transient responses or do not respond to checkpoint blockade. A prerequisite for a vigorous therapeutic response is that the tumor must induce a significant immune response that can then be reinvigorated by checkpoint blockage. Many approaches are being tried in combination with checkpoint blockade to enhance the anti-tumor response. With respect to this proposal, a major goal is to boost anti-tumor responses by increasing the frequency of tumor-specific T cells. Using preclinical models, our approach is to vaccinate tumor-bearing mice with antigens that are unique to the tumor, i.e. tumor neoantigens. These types of antigens are defined by sequencing the expressed genome of the tumor to identify mutations specific to the tumor. A tumors with high numbers of neoantigens correlate with more effective anti-tumor immunity and responsiveness to checkpoint blockade. Notably, current approaches that identify such neoantigens have found that they disproportionately induce anti-tumor CD4+ T cells. Our supporting data show that we can further amplify CD4+ T cell-dependent anti-tumor neoantigen responses by co-administering a novel long-acting form of IL-2, a potent T cell growth and activating cytokine. Vaccination with neoantigens and this form of IL-2 promotes more vigorous anti-tumor CD4+ T cells responses than either agent alone. Based on these findings, our overall hypothesis is that our IL-2- based biologic will amplify neoantigen-specific anti-tumor CD4+ T cells that enhance their susceptibility to checkpoint blockade to lead to a “hot” tumor microenvironment that results in effective anti-tumor immunity. We do not know much about the mechanisms by which these neoantigen-specific CD4+ T cells mediate anti-tumor immunity. Are these anti-tumor responses due to direct antitumor activity by these CD4+ anti-tumor T cells or do they “help” CD8+ T cells, which in turn destroy the cancer cells? We also do not know the extent such anti-tumor responses may be further improved by administering checkpoint blockade. We plan to optimize our approach and address these issues in melanoma and colon cancer models systems. The molecular and cellular mechanisms of these anticancer responses will be studied by focusing on immune cells within the tumor microenvironment. Since clinical trials are ongoing in patients vaccinated with cancer neoantigens, these preclinical studies are designed to set the stage to extend these types of immune-based combination strategies to patients with 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.

4. **Grant #:** 21B04 Elucidating PTEN Tumor Suppression in Melanoma

Principal Investigator: Florian Karreth, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: Phosphatase and tensin homolog (PTEN) is the primary antagonist of the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway and is heterozygously or homozygously lost in >60% of melanoma cases. Notably, activating mutations in PI3K are common in many cancers but rare in melanoma, suggesting that loss of PTEN has more pronounced oncogenic effects in this disease. BRAFV600E-driven melanoma mouse models demonstrated that loss of PTEN potently promotes melanoma development. However, inhibition of the PI3K/AKT pathway had very limited effects on melanoma growth and only slightly enhanced the cytotoxic activity of BRAF inhibition. Importantly, PTEN has additional, lipid phosphatase-dependent and -independent functions. We found that re-activation of PTEN expression alone completely halted tumor growth in a genetically engineered melanoma mouse model. However, it is unclear to what extent these functions suppress melanomagenesis and if restoring complete PTEN activity has superior antitumor activity compared to inhibition of the AKT pathway. We propose to define the therapeutic efficacy of PTEN reactivation in vitro and in vivo relative to AKT or PI3K inhibition, and to measure the synergistic antitumor activity of combining PTEN re-activation with MAPK pathway inhibition. To further elucidate the contribution of the various functions of PTEN to tumor suppression, we will generate BRAFV600E mice that lack expression of endogenous PTEN and harbor an inducible allelic series of PTEN mutants with impaired lipid-phosphatase, protein-phosphatase, or phosphatase-independent functions. Staff will assess the ability of the PTEN mutants to prevent melanoma development, which we will use as a read-out to determine the tumor suppressive activity of individual PTEN functions. We will identify the lipid and protein phosphatase-dependent downstream effectors, both by a candidate approach and unbiased phosphoproteomics, and determine if PTEN downstream effectors suppress melanoma via tumor cell-intrinsic or immune-related mechanisms. Finally, we will perform a inhibitor screen to identify vulnerabilities of melanoma cells that lack PTEN protein or lipid phosphatase activity, with the goal of determining drug combinations with high potency against PTEN-deficient melanomas. It is anticipated that this work will shed light on the complex nature of PTEN-mediated tumor suppression, which researchers will exploit for the rational design of combination therapies to treat PTEN-deficient melanoma.

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 #:** 21B05 Ursolic Acid as a Countermeasure to Cancer Cachexia

Principal Investigator: Andrew R. Judge, PhD**Organization**: University of Florida

Abstract: Cachexia is a devastating catabolic condition characterized by the progressive loss of skeletal muscle mass and body weight which affects up to 80% of patients with cancer. The loss of muscle mass contributes to functional deterioration of both locomotor and respiratory muscles and diminished physical function and quality of life, and 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 responsible for up to 30% of all cancer-related deaths. Unfortunately there is currently no medical therapy for cachexia, which is an enormous unmet need to improve quality of life and enhance survival of cancer patients. In 2011, ursolic acid was discovered to induce an mRNA expression signature which negatively correlated with the mRNA expression signature of atrophying human skeletal muscle in response to spinal cord injury and fasting, suggesting that ursolic acid might counter muscle atrophy. Ursolic acid is a natural compound derived from several edible herbs and fruits, including apples. Since 2011, ursolic acid has been shown to reduce muscle atrophy in rodent models of muscle disuse, fasting, spinal cord injury, chronic kidney disease, and aging but, to our knowledge, has never been tested as a countermeasure to cancer cachexia. However, preliminary data collected for this proposal suggest that ursolic acid can reduce muscle and fat wasting (cachexia) in mice injected with colon 26 adenocarcinoma cells. Based on this exciting preliminary finding, coupled with the favorable safety profile of ursolic acid, with an oral LD50> 8,000 mg/kg and an intraperitoneal LD50> 600 mg/kg in mice, we propose to conduct a pre-clinical trial of ursolic acid in multiple mouse models of cancer cachexia. To do this, we will inject mice with murine or human colon, lung, breast, or pancreatic cancer cells and, once tumors are palpable, treat mice with ursolic acid or vehicle, with or without cancer-specific chemotherapy. With completion of this pre-clinical trial we will have established the extent to which ursolic acid can attenuate cachexia, and improve muscle and respiratory function, in response to four different cancers using murine and human cancer cells in mice which are naïve to, or treated with, chemotherapy. A positive outcome from this work could lead to a subsequent clinical trial for ursolic acid to impede cachexia and improve functional outcomes in cancer patients.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

6. **Grant #:** 21B06 Missing Link between Aging and Lung Tumorigenesis

Principal Investigator: Gina DeNicola, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: Lung cancer accounts for the largest number of cancer-associated deaths in the state of Florida. While great strides have been made due to the introduction of targeted therapies against specific oncogenic drivers, many lung cancer patients do not respond to these treatments or relapse following an initial response. A more comprehensive understanding of the molecular circuits that underpin lung tumorigenesis will spur the development of new and improved therapeutics. Aging is the main risk factor for non-smoking related lung cancer. Surprisingly, the profound metabolic changes accompanying the aging process are rarely considered when attempting to decipher the molecular mechanisms responsible for lung cancer. Our attempt to fill this knowledge gap is focused on pyridine nucleotides including NAD(H) and Nicotinamide adenine dinucleotide phosphate (NADP(H)) which are obligatory coenzymes/cosubstrates for numerous enzymes that play pivotal roles in a host of different cellular processes. Pyridine nucleotide metabolism is a highly-dynamic process involving an intricate network of diverse synthesis, conversion and consumption pathways. An intriguing link between pyridine nucleotides and aging was gleaned by Drs. Gomes' recent discovery that plasma quinolinic acid, an intermediate in the NAD+ de novo synthesis pathway, is increased with aging. In contrast, the putative rate-determining enzyme in the NAD+ salvage pathway, Nicotinamide Phosphoribosyltransferase (NAMPT), which is the focus of Dr. Gardell's research, displays reduced expression with aging. These combined findings suggest that aging may rewire NAD+ production towards its de novo synthesis. Moreover, Drs. Gomes and DeNicola have shown that the NAD+ metabolizing enzymes NADK and Nicotinamide Nucleotide Transhydrogenase (NNT) (cytosolic and mitochondrial, respectively) play crucial roles during distinct stages of tumorigenesis. We aim to illuminate the putative links between aging, NAD+ and lung cancer by leveraging the complementary and overlapping expertise of our research team. We hypothesize that NAD+ biosynthesis is reprogramed during the aging process and, in turn, this maladaptation confers an increased risk for lung cancer. Our collaborative efforts will interrogate the set of enzymes regulating the intracellular levels of pyridine nucleotides using a variety of in vitro (cell culture studies) and in vivo (murine studies) experimental approaches. We will probe the influence of aging, compartment-specific NAD+ metabolism and their ensuing impact on lung tumorigenesis. This proposal holds great promise for identifying new and actionable therapeutic targets for treating lung cancers that do not respond to conventional therapies

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 #:** 21B07 Cholinergic Mechanisms Underlying Cognitive Deficits During and Following Chemotherapy for Breast Cancer

Principal Investigator: Rex Philpot, PhD

Organization: University of South Florida

Abstract: According to the American Cancer Society, breast cancer is the most common form of cancer diagnosis. As of January 2019, approximately 3.8 million women with a history of breast cancer were living in the U.S., and estimates predict 279,100 new cases in 2020 alone. Although breast cancer is the second leading cause of cancer deaths in the U.S., the breast cancer survival rate is fifth highest, with 90% surviving five years or more following diagnosis. As cancer survival rates and longevity continue to increase, the long-term outcomes for breast cancer survivors becomes an increasingly important area of cancer research. Up to 75% of breast cancer survivors have trouble with memory, attention, organization and multitasking as a result of chemotherapy, a phenomenon known as chemotherapy-related cognitive deficits (CRCDs), or "chemo-brain." These deficits persist for decades, interfering with day-to-day functioning and negatively affecting their quality of life. As early detection, diagnostic methods and treatment options continue to improve, breast cancer survival rates will continue to rise, resulting in a greater number of breast cancer survivors that suffer from “chemo-brain.” This makes the treatment of the persistent CRCD’s an increasingly important issue for breast cancer treatment research. Despite this fact, there has been little research on the prevention or treatment of this condition. Several medications have been identified which are useful in improving memory and general mental functioning, but their effectiveness in treating “chemo-brain” has not been examined. The present study will examine the ability of these pharmacological agents to prevent and/or treat the symptoms of “chemo-brain” in an animal model. Using laboratory conditions to identify effective pharmacological agents for the treatment of CRCDs represents a first step toward developing an effective intervention for “chemo-brain,” allowing for the identification of specific targets for prevention and/or treatment of this condition. Based on data from our laboratory, persistent memory deficits occur in tumor bearing mice exposed to the chemotherapeutic agents cyclophosphamide and doxorubicin, allowing us to model CRCDs. The goal of the proposed studies is to provide a proof of concept for the use of nicotinic receptor activators in the prevention and/or treatment of CRCD’s, allowing for future clinical trials in order to prevent the incidence of "chemo brain" and improve quality of life as well as general functioning for 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.

8. **Grant #:** 21B09 Biobanking for Breast Cancer Prevention and Disparity Research in Florida

Principal Investigator: Kathleen Egan, ScD

Organization: H. Lee Moffitt Cancer Center

Abstract: Breast cancer remains by far the leading cancer diagnosis among Florida women and the second cause of cancer mortality after lung cancer. In spite of declining overall death rates, progress has continued to lag in minority and underserved populations. Early detection and

prevention are key to reducing breast cancer incidence and eliminating disparity in breast cancer outcomes. In a collaborative project encompassing major academic cancer centers, we aim to develop a centralized data center and biospecimen infrastructure to support breast cancer prevention research in Florida targeted to screening-aged women. The proposal is to build a comprehensive research infrastructure with standardized collection of epidemiologic data and germline DNA/urine/stool/tissue samples for future breast cancer prevention research. Moffitt Cancer Center is a leader in mammographic breast density determination and Moffitt along with the University of Florida have collaborated over the last several years on studies of the gut microbiome as a novel and modifiable breast cancer risk factor based on mammography patients enrolled at our centers. In this three- year grant, we intend to broaden inclusiveness of the study population and specifically to increase representation of minorities and underserved residents of our state by initiating similar recruitment and data collection protocols at mammography clinics in Miami and Jacksonville that offer large numbers of Hispanic and African American women, respectively. In this three-year project we aim to: Develop methods for outreach and recruitment of minority/underserved women; Enroll ~1,600 women undergoing screening mammography at imaging centers affiliated with three project sites; Collect detailed lifestyle, medical and reproductive histories via an on-line questionnaire; Bank germline oral DNA, urine, stool and biopsy breast tissue (as applicable) samples; Assemble digital imaging data from mammograms and establish a reading center at Moffitt for centralized, standardized evaluation of breast density and novel high-risk mammographic features; Explore and lay ground-work for studies of other promising radiographic screening modalities for early breast cancer detection in high-risk women; Establish a searchable, on-line database for tracking assembled resources, projects, investigators, contacts and funding; and Implement an administrative frame-work for data use and sample sharing with collaborators and independent researchers throughout the state of Florida. The project will improve infrastructure/resources in Florida in the designated areas of tissue banking, medical imaging, and health disparities. The research is responsive to four of the seven research priorities of the latest Bankhead-Coley funding initiative, namely, first, prevention in one of five Bankhead-Coley priority cancers (breast); second, screening and early detection with the inherent goal of identifying high-risk subgroups to whom screening protocols may be tailored to achieve reductions in breast cancer mortality; third, health disparity by contributing to understanding of increased breast cancer mortality in African American women via lifestyle factors and genetic/urine/tissue/gut microbiome biomarkers; and fourth, obesity by contributing knowledge on the relationship of obesity to breast cancer disparity and important breast cancer risk factors including breast density and circulating 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.

9. **Grant #:** 21B10 Mechanisms of Oncogenic Virus-Mediated Chronic Inflammation and Tumorigenesis

Principal Investigator: Noula Shembade, PhD

Organization: University of Miami

Abstract: Oncogenic viruses such as Epstein-Barr virus (EBV), Kaposi sarcoma-associated herpes virus (KSHV), and Human T-lymphotropic virus type 1 (HTLV-1) are responsible for 12-15% of cancers in humans worldwide, including leukemias and lymphomas. HTLV-1, EBV, and KSHV persistently activate Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB), a family of transcription factors with pleiotropic functions. The result of this long-term NF-κB activation is chronic inflammation, which leads to the development of numerous cancers, including leukemias and lymphomas. Viral oncogenes associated with these viruses maintain chronic activation of inflammation via NF-κB, which is a critical early step in inflammation and in the pathogenesis of oncogenic virus infection. Unfortunately, the current treatments used to alleviate or prevent chronic inflammatory related diseases and oncogenic virus-mediated leukemias and lymphomas are highly ineffective; thus, there is a significant demand for novel and more effective therapies. Cells have evolved elaborate negative feedback mechanisms to maintain transient NF-κB activation in response to pro-inflammatory cytokines and microbial infections, which are strongly dysregulated by viral oncogenes. Guanine Nucleotide Exchange Factors (GEFs), which activate small GTPases, are involved in on-off switch mechanisms in a host of signaling pathways, yet their roles in NF-κB signaling pathways are unknown. The surprising finding of our GEFs siRNA library-based screening studies is that TIAM1 (T-Cell Lymphoma Invasion And Metastasis 1) is required for viral oncogenes to maintain the chronic activation of NF-κB. Also, TIAM1 regulates transient activation of NF-κB in cells stimulated with pro-inflammatory cytokines. Our preliminary studies also suggest that the functional specificity of TIAM1 and ability to inhibit or activate NF-κB is dependent on its posttranslational modifications, such as phosphorylation and ubiquitination. In addition, a known TIAM1-associated small GTPase Rac1 is also required for HTLV-1, EBV and KSHV-encoded oncogenes to activate NF-κB. TIAM1 and Rac1 are also involved in the termination of NF-κB signaling following stimulation with pro-inflammatory cytokines. Furthermore, rapid induction of (cell adhesion molecule 1) CADM1 in response to HTLV-1, EBV, and KSHV-infection is strongly associated with TIAM1 and viral oncogenes. Based on these novel results, we plan to identify the mechanisms of TIAM1 and Rac1-mediated aberrant regulation of NF-κB and inflammation. To test this hypothesis, we will use a CADM1, TIAM1, and Rac1 silencing approach in HTLV-1, EBV and KSHV-infected cells and cell lines. Data obtained from this project will reveal the mechanisms of chronic activation of NF-κB in cancers associated with multiple oncogenic viruses, and will also pave the way for therapeutic intervention in these viral-mediated cancers, which are commonly seen throughout the United States.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

10. **Grant #:** 21B11 Impact of the Microenvironment on Breast Cancer Genomic Instability

Principal Investigator: Jerome Irianto, PhD

Organization: Florida State University

Abstract: Among all cancer-related deaths, breast cancer ranks second in women. Because most breast cancer deaths occur in the metastatic stage, a better understanding of the mechanism of metastatic breast cancer is urgently needed and will improve the survival rate. Multiple studies have reported alteration in DNA sequences associated with breast cancer metastasis. These DNA sequence changes, termed mutations, are likely to be selected along with the metastatic progression. Conversely, the mutations being selected are likely to be essential for the process of metastasis itself. Unfortunately, the mechanisms behind the inter-dependency between metastasis and metastasisassociated mutations are unknown. One possible way to elucidate this gap in knowledge is to decouple the multiple factors in the complex metastasis process, including cell migration and change of extracellular microenvironment, and investigate the impact of these factors on mutation rates. Previously, we found mutations can be induced by migrating some cancer cells through small constriction; in addition, matrix stiffness modulates DNA repair and mutation rates. Based on these observations, we hypothesize that metastasis of breast cancer cells and change in microenvironment stiffness contribute to the mutations that are essential to metastatic progression. We will utilize publicly available genomic data from breast cancer patients and quantify the mutations between the primary and distant metastatic tumors. These mutation data will be compared to data from patient-derived tumor cells that migrated in the experimental setting to derive the genomic variations caused by constricted migration. Gene expression profile will be assessed to provide functional correlations. The mutation analysis pipelines have been developed to derive the affected pathways. Next, to directly measure the impact of microenvironment stiffness, cells derived from primary tumors will be cultured in substrates with varying stiffness, and their genomes will be quantified and compared to corresponding patient-derived genomic data. The causal effect of the mutations identified from our research will be validated in an animal model of breast cancer. We will dissect the progression of breast cancer metastasis from the novel perspective of mechanobiology and genomics. The proposed research will expand our fundamental knowledge and mechanistic understanding of metastasis-associated mutations in breast cancer, which is in line with the Discovery Science grant mechanism of the Bankhead-Coley Cancer Research Program. Importantly, our proposed research is highly translational in identifying key regulators that are essential for breast cancer metastasis progression. These key regulators can be targeted therapeutically to improve the survival rate of breast cancer patients. In addition, our research on breast cancer will contribute to the broader cancer field's understanding of how physical interactions between tumor cells and their microenvironments impact tumor cellular functions.

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 #:** 21B12 Non-invasive Adiomic Biomarkers to Predict Treatment Response for Immunotherapy of Lung Cancer

Principal Investigator: Matthew Schabath, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: Cancer immunotherapy (IO) has demonstrated durable clinical benefit in 20-50% patients with advanced stage non-small-cell lung cancer. The patterns of IO patient response are complex, including rapid disease progression and acquired resistance. Because of this complexity, there is a pressing challenge to identify predictive biomarkers that can identify patients that are least likely to respond and may experience rapid and lethal outcomes. Though tumor programmed cell death ligand-1 (PD-L1) expression by immunohistochemistry (IHC) is an approved biomarker, recent published clinical trials demonstrated significant improvements in outcomes regardless of PDL1 expression level. Furthermore, tumor mutational burden (TMB) has been shown to be a superior predictor of IO response. However, there are limitations with TMB as tumor specimens need to be sufficient in quantity and quality. Further, tumors are dynamic and accumulate mutations rapidly and laboratory methods to calculate TMB can be timely and expensive. Moreover, tumor-based biomarkers (such as PD-L1 and TMB) are obtained by biopsy with associated morbidities, and are subject to sampling bias due to the heterogeneity of the biopsied locations. As such, complimentary biomarkers that are predictive, non-invasive, and measured in a timely fashion would have direct translational implications. Quantitative image-based biomarkers (“radiomics”) reflect the underlying pathophysiology and tumor heterogeneity and have many advantages over tissue-based biomarkers, as they can be rapidly calculated from standard-of-care medical images and they reflect the entire tumor and not just the portion of the tumor that is biopsied as in the case for PD-L1 and TMB. Emerging evidence demonstrates the utility of radiomics as a non-invasive approach to predict lung cancer treatment response of targeted therapy, chemotherapy, and stereotactic radiation therapy. Our group has conducted preliminary studies demonstrating that pre-treatment radiomic features combined with clinical data predict rapid disease progression among lung cancer patients treated with IO. Building upon this prior work, the following Specific Aims are proposed to conduct a multi-institutional study to develop and validate radiomic signatures to predict IO treatment response for lung cancer: Aim 1. Assemble a multi-institutional cohort into a clinical-radiomics database of lung cancer treated with IO. Patients from Moffitt Cancer Center, University of Florida, and James A. Haley Veterans' Hospital will be included. Pre-treatment and follow-up CTs and PET/CTs will be retrieved and relevant clinical data elements will be obtained. outcomes. Using an established radiomics pipeline, ROIs will be identified, segmented, and radiomic features will be extracted. Aim 2. Build and validate parsimonious models to predict IO treatment response using radiomic and clinical data. In (2.1) analyze CT radiomics to predict treatment response, in (2.2) analyze PET/CT radiomics to predict treatment response, and in (2.3) fuse CT and PET/CT radiomics to predict response. Baseline peritumoral

radiomics, intratumoral radiomics, and clinical data will be considered and machine learning approaches developed by the team will be used to identify parsimonious models that contain the most informative radiomic and clinical covariates to predict patient outcomes. Patients will be randomized into training, test, and validation sets.

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 #:** 21B13 The Role of ALKBH5 in Leukemogenesis

Principal Investigator: Zhijian Qian, PhD

Organization: University of Florida

Abstract: Acute myeloid leukemia (AML), one of the most common types of leukemia, is a cancer caused by gene mutations and genomic rearrangements in blood cells. A large range of mutations or rearrangements in DNA can cause AML, but some are more commonly observed in AML patients than others. Two of the most frequently observed mutations in pediatric and elderly patients are AML Eighty Twenty One (ETO) (also known as RUNX-ETO) and MLL-rearrangements, which are the result of chromosomal rearrangements. N6-methylation (also written as m6A) is a process where the cell edits DNA or RNA sequences by adding a tag (a methyl group), which can be recognized by other proteins in the cell. Recent evidence has shown that m6A RNA methylation plays an important role in many normal biological processes, as well as cancer development, by providing additional regulation to how and when proteins are expressed in the cell. Currently, the role and underlying mechanisms of m6A modification in leukemia development is not yet understood. m6A RNA methylation is a dynamic process, in which the addition of m6A is carried out by protein complexes (known as m6A methyltransferase complexes) and the removal of m6A is performed by another set of proteins (known as demethylases). One of the proteins involved in removing m6A is ALKBH5, which has been linked toseveral types of solid tumors (e.g., breast cancer and glioblastoma) and promotes self-renewal of cancer stem cells. We found that AlkB Homolog 5, RNA Demethylase (ALKBH5) is upregulated in human AML and that its overexpression is associated with poor prognosis in AML patients. Functional studies showed that ALKBH5 plays a critical role as an m6A demethylase in the development and maintenance of AML caused by a MLL-rearrangement (MLL-AF9) and that it is essential for leukemia stem cell/leukemia initiating cell (LSC/LIC) self-renewal. We hypothesize that ALKBH5 plays a critical role in subsets of AML by regulating protein expression through its demethylase activity. For this reason, it is believed that ALKBH5 may be a common therapeutic target for subsets of AML with MLL-rearrangements and AML-ETO. In this proposal, we will investigate the role of ALKBH5 in the progression of AML induced by AML-ETO, and its role in the maintenance of leukemia stem cells/leukemia initiating cells. Additionally, it will be determined whether and how ALKBH5 regulates different sets of RNA

sequences in subsets of AML, as well as normal blood stem cells. Lastly, it will be determined the key mediators of ALKBH5 in subsets of AML by functional validation studies.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Bankhead-Coley Cancer Biomedical Research Program
Appendix B
Fiscal Year 2020-2021 Active Grants
Funding Fiscal Year 2019-2020

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
20B01	All Children's Research Institute, Inc.	Masanobu Komatsu, PhD	$636,611	4/30/2023	No	No	No
20B03	H. Lee Moffitt Cancer Center	Kenneth H. Shain MD, PhD	$636,610	5/31/2023	No	No	No
20B04	H. Lee Moffitt Cancer Center	Paulo Rodriguez, PhD	$636,610	5/31/2023	No	Yes	No
20B06	H. Lee Moffitt Cancer Center	Andriy Marusyk, PhD	$636,610	4/30/2023	No	No	No
20B07	H. Lee Moffitt Cancer Center	Lixin Wan, PhD	$636,610	5/31/2023	No	Yes	Yes
20B08	H. Lee Moffitt Cancer Center	John M Koomen, PhD	$253,555	5/31/2023	No	No	No
20B10	H. Lee Moffitt Cancer Center	Nicholas J. Lawrence, PhD	$636,610	11/30/2022	Yes	Yes	No
20B11	University of Florida	Elias Sayour, MD, PhD	$636,610	5/31/2023	No	Yes	Yes
20B12	University of Miami	Sabita Roy, PhD	$636,610	5/31/2023	No	No	No
20B13	University of Miami	Jaime R. Merchan. MD	$636,610	5/31/2023	No	No	No
20B14	University of Miami	Marzenna Blonska, PhD	$636,610	5/31/2023	No	No	No
20B15	University of Miami	Luis Morey, PhD	$636,610	5/31/2023	No	Yes	No
20B16	University of Miami	Paulo S. Pinheiro, PhD	$750,000	5/31/2023	No	Yes	No
20B17	H. Lee Moffitt Cancer Center	Jiandong Chen, PhD	$636,610	6/30/2023	No	No	No

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

Principal Investigator: Masanobu Komatsu, PhD

Organization: All Children's Research Institute, Inc.

Abstract: In this reporting period, the research staff analyzed novel signature genes of intratumoral HEVs that were identified in the comparative transcriptome analysis of breast cancer specimens. The laser-capture microdissection followed by RNAseq of tumor-associated HEVs vs. non-HEV tumor vessels revealed 76 differentially expressed genes. Among these, focus was on four genes, TSPAN7, MEOX2, ANKRD53, and ZNF280C and analyzed the expression pattern in the tertiary lymphoid structures (TLS) and non-TLS area of breast cancer. Interestingly, TSPAN7 was expressed in MECA79-positive HEVs as well as in MECA79-negative tumor vessels that are surrounded by lymphocyte clusters, suggesting the existence of MEC79- negative HEV-like vessels. Furthermore, the team analyzed the Cancer Genome Atlas (TCGA) database and found expression levels of TSPAN7 and MEOX2 correlate survival in advanced breast cancer. These findings suggest that the molecular signature of HEVs that were

identified here may be useful for guiding immunotherapies and provide a new direction for investigating tumor-associated HEVs and their clinical significance.

In this period, the team also investigated the potential therapeutic strategy to induce HEV formation in tumors using mouse tumor models. In this study, the team tested a combination of STING and lymphotoxin beta receptor agonists and examine its effect on tumor immune environment. Interestingly, the combination treatment, but not individually, significantly increases tumor infiltration of B cells. STING-alone treatment increased T cell infiltration, but not B cells or TLS-like clustering. LTbR agonist alone increased HEV formation without TLS formation suggesting that increased intratumoral HEV formation alone is not sufficient for TLS formation. The effect of agonist combination was observed in B16F10-OVA melanoma tumors and in MMTV-PyMT mammary tumors. In particular, infiltrating B cells accumulated in TLS-like lymphocyte clusters that also included abundant T cells. There has not been a treatment strategy to induce B cell-containing TLS in mouse tumor models. This is potentially an important breakthrough finding of a way to therapeutically induce immunostimulatory tumor microenvironment.

Follow on Funding: None at time of reporting.

Collaborations: None at time of reporting.

Journals: None at time of reporting.

Patents: None at time of reporting.

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

Principal Investigator: Kenneth H. Shain MD, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: The goal in with this proposal is identify and validate novel protein-specific nuclear export signal inhibitors (NESi) to prevent XPO1 binding with TOP2A in order to maintain it in the nucleus; thereby, (1) sensitizing MM cells to topoisomerase inhibitors and (2) the developing a new class of drugs for MM and other malignancies. It is proposed the following aim to carry-out this goal.

Aim 1: Synthesize new NESi that block TOP2A-XP01 binding and characterize their activity. Our team has identified a total of 100 additional compounds with potential binding to the crystal structure of TOP2A. From this list, 20 compounds have been identified for screening in our functional assays.

Aim 2: In collaboration with investigators at the University of Florida, use the crystal structure of TOP2A to select additional NESi by screening large chemical libraries in silico. In silico examination of, 1,595,088 molecules identified and additional 100 compound including the highest scoring compound was 5-(1H-Imidazol-2-yl)-N-methyl-N-[(3R)-1-(3-phenylpropyl) piperidin-3-yl]thiophene-2-carboxamide. We are curating this list for compounds with the highest

potential for further testing in our screen and, in turn, selected compounds will be utilized for further testing of TOP2A NESi function and activity.

Aim 3: Optimize and define the activity of the NESi discovered in Aims 1 and 2. One of the major aspects of Aim 3 is a develop a high through put assay system to screen TOP2A-XPO1 complexes. We have made significant progress on the development of an additional (potentially alternative) functional screening method. This platform will (1) increase our capacity to screen agents alone and in combination with doxorubicin in cell lines using a 1536-well plate robotic liquid plate handler (127 compounds simultaneously), (2) determine synergy between agents and (3) will also facilitate examination in patient specimens (MM, NHL and ALL) for ex vivo validation (see below). As an example, testing of the first 5 patient samples (of 50) with NSC-9138 and doxo demonstrated synergy in 60%. Additionally, these ex vivo results are linked to RNA sequencing and whole exome sequencing (WES) to identify companion biomarkers associated with activity.

In summary, the multi-disciplinary team has successfully identified, developed and validated a novel protein-specific NESi. Research staff has successfully identified and demonstrated topo-specicfic NESI activity of our lead compound NSC-9138. This work was presented in abstract form at the AACR 2021 meeting and the final drafts for submission of our first joint manuscript entitled: Novel small molecule inhibitors that target the exportin binding pocket of TOP2A for the treatment of multiple myeloma. Collectively, the reseach teams' findings have established (1) a novel TOP2A-specifc NESi capable of sensitizing MM cells to topoisomerase inhibitors and (2) a platform for the development of a new class of drugs for MM and other malignancies. Our preliminary has suggested that NCI-9138 has activity in additional cancer models included AML, NHL and breast cancer. We expect that our findings will lead to high impact manuscripts, federal grants and the establishment of novel class of protein-specific NESi with broad applicability to patients in Florida and elsewhere with diverse cancers.

Follow on Funding: None at time of reporting.

Collaborations: Within the context of this FDOH grant we are actively collaborating with intra-Moffitt as well as our colleagues at the University of Florida (Dr. David Ostrov; Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine). Dr. Ostrov we have carried out the first steps in our proposed structure-based strategy to select small molecules that bind human topoisomerase IIα (TOP2A) nuclear export signal (NES) sites (site A). Intra-Moffitt collaborator (Dr. Nicholas Lawrence) has been critical in evaluating and synthesizing compounds for further screening and investigation.

Journals: None at time of reporting.

Patents: None at time of reporting.

3. **Grant #:** 20B04 Notch Signaling Boosts T cell-based Immunotherapy

Principal Investigator: Paulo Rodriguez, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: Development of immunotherapies based on the transfer of tumor-reactive engineered-T cells has emerged as a promising treatment for cancer. However, the immunosuppressive nature of the tumor microenvironment (TME) represents a major limitation for the effectiveness of anti-cancer T cells. Throughout this grant, the focus is to develop new strategies that make T cells resistant to the regulatory TME and that will increase the effectiveness of therapeutic T cells in cancer by promoting activation of Notch signaling. This strategy is likely to benefit Floridians with melanoma and lung tumors by enabling efficient cellular-based therapies.

The research team aimed to elucidate the effect of the modulation of Notch in the activity of tumor-specific T cells by culturing melanoma-gp100-specific T cells (Pmel) in the presence of plate-bound Notch-ligand wild-type-DLL4 (WT-DLL4) or high-affinity variant DLL4 with a higher capacity to prime Notch (DLL4.v3). Results show higher production of T cell effector molecule IFNg, but not activation or differentiation, in pmel T cells primed under plate-bound DLL4.v3 compared to controls, indicating that Notch signaling promotes effector molecules in T cells.

To establish the role of Notch signaling in human T cells, a model was created whereby human T cells are activated and co-cultured with artificial-antigen presenting cells (aAPC) transduced with viral vectors encoding DLL4.v3, WT-DLL4, or empty vector. Enhanced proliferation and higher production of IFNg were found in primed T cells co-cultured with DLL4.v3-aAPC compared to counterparts exposed to aAPC-WT-DLL4 or aAPC-mock cells. Also, we established the conditions for the transduction of human T cells with anti-tumor chimeric-antigen receptors (CAR, FSH-receptor) or T cell-receptor (NY-ESO-TCR). Results support the development of models to test the activity of engineered T cells.

The staff continue assessing the effects of co-culturing therapeutic T cells with aAPC expressing DLL4.v3 and found promotion of additional cytotoxic drivers. Also, a model was established to assess the specific elimination of tumors by T cells expressing CARs against FSH-receptor or NY-ESO-TCRs, both allowing recognition and elimination of cancer cells. This model enabled to test effect of Notch signaling by DLL4.v3 in the cytotoxicity of engineered-T cells.

The team tested the effect of the promotion of Notch in FSH-CER transduced T cells. Higher anti-tumor effect was elicited by FSH-CER T cells against FSHR+-OVCAR3 cells expressing DLL4v3 compared to counterparts exposed to control tumor cells. Also, the team tested the anti-tumor actions of a fusion protein that contains the anti-PD-L1-scFv fused to DLL4.v3. This compound induced significant anti-tumor effects in mice and promoted tumor-reactive T cells. These results indicate that promotion of Notch in CAR-T cells enhances their capacity to kill tumor cells.

In summary, the team's accomplishments, together with the contribution of the covered key personnel favorably impacted the progression of the study. Continuation of this research is expected to have an impact in the health of Floridians by developing the next generation of effector T cells for cancer treatments.

Follow on Funding: None at the time reporting.

Collaborations: None at time of reporting.

Journals: Verma V, Jafarzadeh N, Boi S, Kundu S, Jiang Z, Fan Y, Lopez J, Nandre R, Zeng P, Alolaqi F, Ahmad S, Gaur P, Barry ST, Valge-Archer VE, Smith PD, Banchereau J, Mkrtichyan M, Youngblood B, Rodriguez PC, Gupta S, Khleif SN. MEK inhibition reprograms CD8+ T lymphocytes into memory stem cells with potent antitumor effects. Nat Immunol. (2020). Pubmedid: 33230330.

Tumor-related stress regulates functional plasticity of MDSCs. Mandula JK, Rodriguez PC. Cell Immunol. 2021 May;363:104312. doi: 10.1016/j.cellimm.2021.104312. Epub (2021) PMID: 33652258

Patents: None at time of reporting.

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

Principal Investigator: Andriy Marusyk, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: The research team developed reliable segmentation pipeline to generate digital mask from high resolution images with histological IHC data.

We have developed and tested multiple spatial analysis metrics capable of uncovering the spatial relationship; currently performing sensitivity and robustness analyses to select optimal analytical pipeline.

We have developed methodology to generate optimal in silico images with known spatial distributions to enable comparison of the alternative approaches.

We have developed initial version of the agent based modeling to interrogate the impact of stromal protection on proliferation/death dynamics in situ.

The research team has generated data for evaluating relevance of 2D based metrics towards understanding the topology in 3D tissues. The team has managed to overcome a key challenge, of proper alignment of consecutive slides, using an approach developed within Moffitt's IMO department (manuscript under review). Now, we are focusing on overcoming more minor challenges in 3D tissue reconstruction, which should enable the team to measure distances within 3D, to compare with 2D inferences.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at time of reporting.

Patents: None at time of reporting.

5. **Grant #:** 20B07 Characterizing Oncogenic Function of ITCH in Melanoma

Principal Investigator: Lixin Wan, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: The team found that the Itchy E3 Ubiquitin Protein Ligase (ITCH) gene contributes to the vemurafenib-resistatance in v-raf murine sarcoma viral oncogene homolog B1 (BRAF)-mutated melanoma cells through activation of the Rapidly Accelerated Fibrosarcoma (CRAF) protein and the oncogenic mammalian target of rapamycin (mTOR) pathway.

Furthermore, the team found that ITCH positive regulates the BRAF oncogenic pathway in several different melanoma and non-small cell lung cancer cell lines with distinct genetic backgrounds. These findings indicate that the molecular module we identified plays a crucial role in the survival of a wide spectrum of tumor cells. The research team also pinpointed the key amino acids in the CRAF protein that are subjected to the regulation by the ITCH enzyme.

Since ITCH has been shown to play an important role in the immune system. Through innoculating mouse melanoma cells into Itch wild-type and knockout mice, we observed a decrease tumor growth of melanoma cells in Itch knockout mice. This observation suggests that ITCH has an immunogenic role in the melanoma immune microenvironment.

Following this direction, we profiled the immune cells populations from the melanoma tumors developed in Itch wild-type and knockout mice. The results revealed increased natural killer cells, cytotoxic T lymphocytes and decreased macrophages in the tumors from Itch knockout mice. Natural killer cells, cytotoxic T lymphocytes have anti-tumor function while macrophages usually help tumor survival.

The research team also generated genetically engineered mouse models to study the role of ITCH in melanoma initiation and progression. Results from a pilot experiment suggest that Itch knockout slowed down the tumor formation in this melanoma mouse model. In an effort to search small molecules that could suppress ITCH function, in collaboration with Dr. Cai's group in USF, we discovered one lead compound that efficiently disrupts ITCH's binding with its substrate.

Follow on Funding: NIH/NCI, Characterizing oncogenic function of ITCH in melanoma, Lixin Wan, $1,888,755.

Collaborations: University of South Florida, Department of Chemistry, Dr. Jianfeng Cai. Dr. Cai is collaborating with us to design/screen compounds that could inhibit or destabilize the ITCH protein.

Journals: Ullah, R., Yin, Q., Snell, A.H., and Wan, L. (2021). RAF-MEK-ERK pathway in cancer evolution and treatment. Semin Cancer Biol (2021). PMID: 33992782 DOI: 10.1016/j.semcancer.2021.05.010

Patents: None at time of reporting.

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

Principal Investigator: John M Koomen, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: Lung cancer metastases respond differently to treatment; progression and regression can be simultaneously observed in different lesions in the same patient. Progression at one site can lead to discontinuation of therapy, so heterogeneity creates a major barrier to patient care. Metastatic heterogeneity is poorly understood; differences include clonal evolution, differential mutation status, and metastatic microenvironments. To study metastatic lung tumors and elucidate factors involved in therapeutic response, our rapid tissue donation after autopsy program supports collection of lung tumors, metastases, and tumor-adjacent tissues as well as extensive medical histories for each patient. Expression proteomics and highly characterized targeted biomarker assays quantify cancer signaling and immune checkpoint proteins in these tumors, which can be correlated with therapeutic response.

The research staff optimized methods for processing formalin fixed paraffin embedded tissue sections and laser capture microdissection of tumor or adjacent tissue using a hematoxylin and eosin stained slide as a reference. Tissue areas are recorded prior to resolubilization of tissue. After Bradford assays, protein digestion and trypsin digestion were performed using filter-aided sample preparation. Proteolytic peptides were analyzed with LC-MS/MS as a digestion quality control step (label-free mini-proteome) prior to Tandem Mass Tag (TMT)16 labeling, label verification with LIquid Chromatography with tandem mass spectrometry (LC-MS/MS), peptide fractionation with bRPLC, and LC-MS/MS. Targeted assays were performed using the same digests with a panel of liquid chromatography-multiple reaction monitoring mass spectrometry assays. Database searches were performed with MaxQuant; data analysis was performed with Perseus as well as an in-house pipeline for TMT analysis prior to pathway mapping.

Pathology review was completed for each slide of tumor tissue and tumor adjacent tissue controls for each metastatic site. Laser capture microdissection was used to isolate each type of tissue. Square areas for tissue recovery were calculated to estimate total protein, which showed reasonable agreement with Bradford assays, Nanodrop, and colorimetric tryptic peptide assays (Pierce). LC-MS/MS quality control identified about 3,000 proteins in each sample (based on ~14,000 peptide identifications per sample). Initial unsupervised clustering and principal component analysis separated the tumors from cell line controls and the tumor adjacent control tissues. Based on this review, each sample was labeled with TMT 16plex reagents. After labeling quality control, samples were combined for offline peptide separation using basic pH reversed phase liquid chromatography with fraction concatenation. MaxQuant analysis of the LC-MS/MS TMT16 expression proteomics dataset identified and quantified more than 7,100 proteins. Statistical analysis and pathway mapping are used to highlight differences between tumor and adjacent tissues, tumors from different patients, and different metastatic lesions from within the same patient. Results have been generated for different tumors types, including those driven by Kirsten rat racroma viral oncogene homolog (KRAS) mutations and Anaplastic lymphoma kinase (ALK) fusions. LC-MRM quantification of cancer signaling proteins and

immune oncology targets complements the expression proteomics results. Data can also be correlated with response to therapy as measured by tumor changes in radiology images to link the proteogenomics data to patient outcomes. Building on this method and example data, we will be able to study other patients in our lung cancer cohort.

Follow on Funding: None at time of reporting.

Collaborations: None at time of reporting.

Journals: None at time of reporting.

Patents: None at time of reporting.

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

Principal Investigator: Nicholas J. Lawrence, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: The aim of the study is to develop new drugs to treat myeloproliferative neoplasms (MPNs) which are a type of blood cancer including chronic leukemias, polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF). The majority of MPNs are driven by mutations in a protein named Janus kinase 2 (JAK2). For this reason, the research is focused on developing new and improved drugs to target JAK2 for treating these blood cancers. Earlier research findings from the team have shown that simultaneous targeting of JAK2 and another cancer-causing protein target called BRD4 leads to better efficacy and outcomes in cellular and animal studies for treating myeloproliferative neoplasm (MPNs). This approach also delays the onset of resistance to the drugs, a common problem with targeted cancer therapies.

The proposed studies will directly impact the health of cancer patients in Florida. The research team will develop novel preclinical compounds targeting cancers that have evaded effective therapy. The team comprises experts in medicinal chemistry and cancer biology to develop and thoroughly characterize novel multi-targeted drugs designed to specifically target cancer signaling events mediated by JAK2 (AIM2) and epigenetic modulators (AIM1). Given the estimated number of people in the U.S. suffering with an MPN it is estimated that over 22,000 Floridians are currently afflicted with an MPN. The team's proposed studies could lead to a direct effect on the healthcare treatment, and improve the lives and life expectancy, of this significant population of Florida.

In the first year of the project the research team has been assembled and initiated the discovery of new small molecules targeting both JAK2 and BRD4. The research team has a new methodology for the large-scale production of JAK2 from mammalian cells, which has facilitated the determination of the first crystal structures of JAK2 bound to the clinically approved JAK2 inhibitors ruxolitinib and fedratinib. Along with biochemical and cellular data, the results provide a comprehensive view of the shape complementarity required to achieve highest activity, which will facilitate the development of more effective JAK2 inhibitors as therapeutics as the project

proceeds. Early compounds in the study can significantly reduce the growth of blood cancer cell lines that depend on the function of JAK2. Compounds have also been developed to degrade JAK2 and are currently under study in multiple blood cancer cell lines. Structural and biochemical characterization of these bi-valent inhibitors will be undertaken in the second year of the project.

The proposed research will provide research tools and scientific knowledge for other investigators and clinicians to use in basic research programs that address the role of JAK2 and BRD4 in MPNs and other cancers. The proposed research is translational and will result in the development of new drugs for MPNs and other cancers. Moffitt and the PIs of this study have a proven track record in partnering with biotech and pharmaceutical companies to translate new preclinical inhibitors. Therefore, translation of the team's studies to patients will lead to a significant impact on the healthcare and lives of MPN patients in Florida.

Follow on Funding: None at time of reporting.

Collaborations: None at time of reporting.

Journals: Structural Insights into JAK2 Inhibition by Ruxolitinib, Fedratinib, and Derivatives Thereof, Ryan R. Davis, Baoli Li, Sang Y. Yun, Alice Chan, Pradeep Nareddy, Steven Gunawan, Muhammad Ayaz, Harshani R. Lawrence, Gary W. Reuther, Nicholas J. Lawrence, and Ernst Schönbrunn, Journal of Medicinal Chemistry, (2021). DOI: 10.1021/acs.jmedchem.0c01952; PMID: 33570945

Patents: E. Schonbrunn, N. J. Lawrence, H. R. Lawrence, BRD4-kinase inhibitors as cancer therapeutics, US patent application No. 63/178,363 (Moffitt ID No. 21MA004PR2) filed 4/22/21

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

Principal Investigator: Elias Sayour, MD, PhD

Organization: University of Florida

Abstract: Despite multimodality approaches for osteosarcoma (OS), including chemotherapy and limb amputation, a significant percentage of children/adolescents succumb to disease due to the presence of lung metastasis; these outcomes necessitate development of novel targeted therapeutics. Immunotherapy promises to redirect the host immune system against OS but remains limited by the dearth of antigen specific targets and the immunosuppressive tumor microenvironment. To circumvent the lack of OS specific targets and overcome intratumoral immunosuppression, our group has developed a novel treatment platform that consists of clinically translatable nanoparticles (NPs) combined with personalized tumor derived mRNA. These RNANPs can simultaneously function as both a vaccine and an innate immunomodulating agent to reprogram OS mediated immunosuppression into an immune activated milieu. We have shown that intravenous administration of tumor mRNA loaded NPs transfect antigen presenting cells and lead to an activated T cell response for induction of antitumor immunity in preclinical models. In contrast to other vaccine formulations, RNANPs

recruit multiple arms of the immune system (i.e. innate and adaptive), and remodel the systemic/intratumoral immune milieu, which remain potent barriers for vaccine, cellular, and checkpoint inhibiting immunotherapies. In murine pulmonary OS models, RNANPs induce robust antitumor efficacy (~87.5% long-term survivor benefit) and mediate synergistic activity in settings where immune checkpoint inhibitors (i.e. antiPDL1 therapy) do not confer therapeutic benefit. Drs. Sayour and Heldermon will explore mechanisms of treatment resistance in syngeneic immunocompetent murine models for metastatic OS (MOSJ and K7M2). They will then use the nanoparticle delivery strategy to target identified mechanisms before pursuing a translational canine study exploring the safety and activity of combination RNANPs in canines with OS (nearly 100% homologous to the human form of the disease).

Follow on Funding: Rally Foundation renewal grant, Elias Sayour, $50,000.

Collaborations: None at time of reporting.

Journals: Melnick K, Dastmalchi F, Mitchell D, Rahman M, Sayour EJ. Contemporary RNA Therapeutics for Glioblastoma [published online ahead of print, 2021 Jun 8]. Neuromolecular Med. (2021) doi:10.1007/s12017-021-08669-9

Patents: None at time of reporting.

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

Principal Investigator: Sabita Roy, PhD

Organization: University of Miami

Abstract: The research team had previously demonstrated that mice with metastatic cancer are associated with severe pain similar to what is observed in humans where 72% of cancer patients particularly patients with metastatic cancer suffer from pain, with a mean intensity of 6.4 (0–10 numerical rating scale). Pain level six is intense pain, that is strong, deep and piercing, dominate your senses, causing you to think unclearly, trouble holding a job or maintaining normal social relationships. Therefore, controlling pain is an essential part of cancer treatment. The most common analgesics prescribed for moderate to severe cancer pain are OPIOIDS. Opioids use has been shown to induce microbial dysbiosis and systemic inflammation. The major focus of the study this funding period is to establish is microbial dysbiosis contributes to metastatic cancer pain. In the previous progress report the team developed a model for metastatic cancer pain. In the current progress report the team report microbial analysis using 16S sequencing in this model of cancer pain.

Research shows luminal contents from the cecum of cancer bearing mice show a distinct pattern from control mice. Statistical analysis using unifrac distance show a significant increase in bacteriodetes and a significant decrease in actinobacteria at the phylum level. At the family level cancer bearing mice show a significant increase in bacteroideace and a decrease in clostridales and Enterobacteriaceae. The decrease in clostridiales is particularly important. Clostridium belonging to family Clostridiaceae and order Clostridiale is the oldest known

thermophilic ethanologen. Special features of these species include co-fermentation of both pentose and hexose sugars, high ethanol yield, cellulolytic activity, and acquiescent to genetic modification.

There probiotic commensals are important in the digestion of complex dietary fibers and for the production in short chain fatty acids such as butyrate. Commensal Clostridia is important for the maintenance of overall gut function. In the next cycle the team will further investigate the role of a decrease in clostridiales in cancer induced dysbiosis and its contribution to cancer pain.

Follow on Funding: None at time of reporting.

Collaborations: None at time of reporting.

Journals: None at time of reporting.

Patents: None at time of reporting.

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

Principal Investigator: Jaime R. Merchan, MD

Organization: University of Miami

Abstract: The main purpose of this project is to develop novel biotherapies for advanced colorectal cancer (CRC) using measles virus (MV) based combination therapies targeting tumor and stromal components. The research team has developed novel measles virus vectors, which are able to target tumor stroma, as well as murine targeted vectors, allowing the team to characterize the effects in syngeneic, immunocompetent colorectal cancer models, in addition to human CRC models. During the 2020-2021 grant period (ending June 30, 2021), significant progress has been made towards aim 1 and started experiments for aim 2, in spite of a temporary delay in initiating the experiments in June 2020 (due to COVID institutional restrictions and lab staff developing COVID-19). The in vitro experiments for aim 1 have been mostly completed, including the in vitro cytotoxicity experiments to determine the cancer cell sensitivity to the non-targeted and re-targeted MV vectors, triptolide, and combinations, effects of the combination on viral replication, and studies to characterize the interactions among virus, tumor cells and stromal (endothelial cells and fibroblasts) cells. In addition, in vivo studies using triptolide in human CRC models have been performed to characterize the in vivo antitumor activity of this novel agent. The main results include the confirmation of the potent effects of measles virus vectors and triptolide against human and mouse CRC cells, the successful replication of viruses in CRC cell lines, and the significant improvement in the tumor killing activity when the virus and triptolide are used in combination, compared to each agent alone. In addition, it was found that cancer associated fibroblasts (CAFs) are permissive to measles virus infection and replication, and importantly, that fibroblasts successfully transfer viral infection to cancer cells in co-cultures, and that infected fibroblasts may serve as a “reservoir” of virus over longer periods of time. This results into a more prolonged viral infection to cancer cells, leading to more efficiently antitumor effects in co-culture compared to infection of single cells alone. The

implications of these findings are significant, as this is first time demonstration of the ability of cancer stromal cells to transfer oncolytic viruses to tumor cells, leading to enhanced CRC killing. Finally, during this period, the in vivo efficacy of minnelide, a novel antitumor agent, was confirmed in vivo, in the HT-29 human CRC xenograft model, where minnelide had a dose dependent antitumor effect against human CRC, leading to significant improvement in overall survival in treated mice. In summary, significant progress has been made in the first year of the grant tenure, and the current results confirm that successful stromal targeting by oncolytic viruses leads to prolonged and enhanced antitumor effects in CRC. This may open new avenues of research, focusing on targeting the stromal components in colon tumors, by oncolytic viruses or other biotherapies. The confirmation that minnelide induces significant in vivo antitumor effects in human colon cancer brings is encouraging and provides a novel treatment option that can be used in combination with oncolytic viruses in colon and other cancers for Floridians and Americans.

Follow on Funding: None at time of reporting.

Collaborations: None at time of reporting.

Journals: None at time of reporting.

Patents: None at time of reporting.

11. **Grant #:** 20B14 Elucidation and Targeting of Novel Molecular Determinants of Tumor Progression and Dissemination

Principal Investigator: Marzenna Blonska, PhD

Organization: University of Miami

Abstract: The molecular and genetic features that drive aggressive clinical behavior of lymphoma (blood cancer) cells have not been fully defined. Moreover, there are very limited data regarding the mechanisms that promote the spread of cancer cells to different organs. Recently, high levels of a protein called forkhead box C1 (FOXC1) have been detected in patients with breast cancer, melanoma, lung cancer, and many other aggressive tumors. Elevated FOXC1 is associated with metastatic disease and poor prognosis. Our recent study demonstrates, for the first time, that FOXC1 can be detected in lymphoma cells, mainly in patients with multi-organ involvement. It further suggests that FOXC1 promotes colonization of distal organs and supports tumor growth in the new environment. To test our hypothesis, the research team generated transgenic mice with the inducible expression of FOXC1 in different tissue types. To provide experimental evidence that FOXC1 promotes the dissemination of lymphoma cells (hematologic tumor) and breast cancer (solid tumor), mouse models of both diseases was created. The experimental animals will be observed and analyzed for a period of 15 months.

The second aim is to identify the genes controlled by FOXC1 in lymphoma and solid tumors. The team searched the public repositories and collected large data sets (over 400 tumor biopsies). The team was interested in lymphoma, breast cancer, lung cancer, and melanoma.

Based on the relative expression of FOXC1, the patients were stratified into two groups: FOXC1high and FOXC1low. Our results from the lymphoma biopsies indicate that FOXC1 regulates genes that facilitate cell migration, invasion, adhesion, and interaction with the tumor microenvironment. The team has also completed the part of the project aiming to reveal the mechanism of aberrant expression of FOXC1. The team found that high expression of FOXC1 correlates with low methylation of the FoxC1 promoter (part of DNA). Changes in DNA methylation affect the Foxc1 promoter accessibility for specific transcriptional activators.

Currently, the team is in the process of identifying and validating compounds that suppress the activation of FOXC1 (potential inhibitors of FOXC1) in tumor cells. During the first year of this grant, the team identified and selected top candidates for translational research.The study will provide a rationale for screening lymphoma patients for elevated FOXC1. It will also help with designing a novel therapy for patients with metastatic tumor.

Follow on Funding: None at time of reporting.

Collaborations: None at time of reporting.

Journals: None at time of reporting.

Patents: None at time of reporting.

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

Principal Investigator: Luis Morey, PhD

Organization: University of Miami

Abstract: Significant progress has been made in all three aims. The research team has discovered new Polycomb-mediated epigenetic mechanisms in estrogen receptor positive breast cancer. Briefly, the team found a new way that the breast cancer cell uses to keep proliferating aberrantly and therefore generating tumors. Moreover, the team has established a strong functional link between Polycomb and resistance to current therapies aimed to target estrogen receptor positive breast cancer, and the team has discovered where this major epigenetic machinery is recruited in the human genome when breast cancer cells become resistant to therapies. These results will shed light into the discovery of potential new therapeutic options for patients with breast cancer that do not respond to current therapies. Notably, some of these findings are now currently under a second round of revision in the prestigious journal Nucleic Acid Research-NAR, (Impact factor 15). In the second year of the award, the team will keep working on the proposed plans and plan to publish at least one more article. Finally, during the first year of the award the team presented in multiple occasions the work derived from this grant in both national and international meetings.

Follow on Funding: None at time of reporting.

Collaborations: Dr. Fenghua Yuan from the lab of Dr. Zhang. Department of Biochemistry & Molecular Biology, University of Miami Miller School of Medicine, Miami, FL 33136, USA

Tong Liu from the lab of Dr. Wang. Department of Computer Science, University of Miami, 1365 Memorial Drive, P.O. Box 248154, Coral Gables, FL, 33124, USA

Dr. Stransky from the lab of Dr. Simone. Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461, USA.

Journals: Liliana Garcia-Martinez, Yusheng Zhang, Yuichiro Nakata, Ho Lam Chan, Lluis Morey. Epigenetic mechanisms in breast cancer therapy and resistance. Nature Communications. (2021). PMID: 33741974

Patents: None at time of reporting.

13. **Grant #:** 20B16 Risk, Etiology, and Mortality for Highly Fatal Cancers in Diverse Florida; Unique Impact on African Americans, Afrocaribbeans, Cubans, Puerto Ricans and Other Hispanics

Principal Investigator: Paulo S. Pinheiro, PhD

Organization: University of Miami

Abstract: The aims of the current research project are to identify critical points in disparities in risk (incidence) and survival for two highly fatal cancers (lung and liver) among the different racially-detailed populations of Florida (e.g. Cubans, Puerto Ricans, Afro-Caribbeans, Whites, African Americans, etc.). The disparities in relation to etiology for lung (e.g. smoking) and liver (e.g. liver hepatitis) among these racial-ethnic populations are unknown in Florida (and elsewhere); therefore, hindering efforts to properly control and prevent these malignancies. The current project has completed its first year, finally managing to assemble some of the key data from the Department of Health dependencies after major delays. Florida Cancer Data System requests are currently taking a minimum of nine months to one year to complete. Additional datasets/authorizations were requested from the Florida Department of Health's Sexually Transmitted Disease Section; the Sylvester Cancer Center and the University of Miami Hospital (data 100% received and being analyzed); Jackson Memorial Hospital (data not received, delays in administrative authorizations due to COVID-19 priorities). Since only partial data is available, only preliminary findings (initial results), can be reported, which should be interpreted with caution.

In regards to lung cancer, the research team has been able to assess patterns in lung cancer, as a whole, and in never smokers. Lung cancer risk is high among non-Hispanic Whites, African Americans, and Cubans in Florida but relatively low-risk in other populations (Haitians); thus, reflecting the differences in smoking prevalence in each population. Lung cancer in never smokers, however, is an important disease, especially in women and ethnic minorities. Precision medicine therapies are also being studied for Florida metastasized patients. Targetable mutations, which benefit from existing effective treatment, are being found to be more common among non-smokers, women, and ethnic minorities. No initial disparities in receipt of testing and corresponding drug administration have been detected.

In regards to liver cancer, very distinct patterns by etiology (alcohol, viral hepatitis, or metabolic disease) have been found for different racial-ethnic groups in Florida. This suggests that

prevention, screening, and clinical surveillance may be better tailored according to race-ethnicity. There is a high incidence of hepatoma (main form of liver cancer) associated with Hepatitis C virus (HCV) among the Puerto Rican population and among US-born African Americans. These populations will potentially benefit from HCV screening (and subsequent treatment if tested positive) in the asymptomatic adult Florida population. The same applies to Haitians but for Hepatitis B-related hepatoma. Metabolic hepatoma is more common among females and Hispanics, in general, while alcohol-related hepatoma is a problem for Hispanic males.

Based on these promising results, an editorial has been published by the PI Dr. Paulo S Pinheiro in the high impact factor Journal of the National Cancer Institute, highlighting the need for results/studies such as this one, as the US population becomes more diverse. Analytical work will be intensified as the final data comes in, publications will be submitted, and the community outreach team will be involved for better dissemination of findings.

Follow on Funding: None at time of reporting.

Collaborations: The University of Miami (UM)-Department of Public Health Sciences (Miami, FL) and Florida A&M University (FAMU)-Institute of Public Health, College of Pharmacy and Pharmaceutical Sciences (Tallahassee, FL) have been fully engaged in the data requests and institutional review board approvals in order to gain access to the required public datasets in Florida.

Ms. Kamaria Jacobs, FAMU doctoral candidate.

Ms. Qinran Liu, UM doctoral student in epidemiology.

Journals: None at time of reporting.

Patents: None at time of reporting.

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

Principal Investigator: Jiandong Chen, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: In the past year, the research effort to develop p53 inhibitors for improving the safety of cancer chemotherapy has been directed to several areas: Synthesis of derivatives of an in-house developed lead compound (#133) to identify more potent analogs, and understand the structure-activity relationship of the lead compound through modifications. These efforts have produced a small library of compounds. Functional analysis performed to date have not yet identified superior derivatives. Additional work is ongoing in this area to complete the analysis of new compounds already synthesized. Re-synthesis of lead compound NSC194598 and improvement of solubility. The research team's major published lead compound NSC194598 has poor water solubility that limits its use in animal and certain biochemical experiments requiring high concentrations. The team has established synthetic scheme to re-synthesize highly pure NSC194598, which provided important validation of the activity observed using stored compound provided by the NCI that contain

impurities. Furthermore, several derivatives of NSC194598 have been synthesized, including two with significantly improved solubility. Importantly, the soluble derivatives showed similar p53 inhibition potency as the parent NSC194598 compound, facilitating experiments on the mechanism and functional studies. Investigation of the mechanism of p53 inhibition by NSC194598. Since this is the most potent and interesting p53 inhibitor identified to date, the has devoted significant effort to further understand how this compound inhibits p53 DNA binding. To this end, the co-investigator on the project has been studying the interaction of NSC194598 and derivatives with p53 using nuclear magnetic resonance (NMR) technology, and also collaborating with an X-ray crystallography group at Moffitt to attempt to co-crystalize the compound with p53 in order to determine the structural basis of the inhibition. The team has started a new round of high-throughput screen to identify new p53 regulators using a cell-based assay. To date ~10,000 compounds have been screened and 42 initial hits have been selected for further testing in the lab. The team plans to continue the screening campaign to test >100,000 compounds.

Follow on Funding: None at time of reporting.

Collaborations: None at time of reporting.

Journals: None at time of reporting.

Patents: None at time of reporting.

Bankhead-Coley Cancer Biomedical Research Program
Appendix C
Fiscal Year 2020-2021 Active Grants
Funding Fiscal Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
9BC01	Florida Atlantic University	Esther A. Guzmán, PhD	$801,000	5/31/2022	No	Yes	Yes
9BC03	Florida State University	Jennifer L. Steiner, PhD	$732,238	5/31/2022	No	No	No
9BC04	Florida State University	George S. Rust, MD, MPH	$800,487	5/31/2022	No	Yes	No
9BC07	H. Lee Moffitt Cancer Center	Gina M. DeNicola, PhD	$1,335,000	5/31/2024	No	No	No
9BC08	H. Lee Moffitt Cancer Center	Nelli Bejanyan, MD	$1,335,000	3/31/2024	No	Yes	No
9BC09	H. Lee Moffitt Cancer Center	Ernst Schonbrunn, PhD	$800,454	3/31/2022	Yes	No	No
9BC12	University of Miami	Anthony J. Capobianco, PhD	$801,000	4/30/2022	Yes	Yes	No
9BC13	University of Miami	Kerry L. Burnstein, PhD	$801,000	4/30/2022	No	No	No
9BC14	University of South Florida	Hong Yuan (Rays) Jiang, PhD	$801,000	4/30/2022	No	Yes	Yes

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

   Principal Investigator: Esther A. Guzmán, PhD

   Organization: Florida Atlantic University

   Abstract: The project seeks to identify marine natural compounds with the novel activity of inducing programmed cell death in triple negative breast cancer (TNBC) cell spheroids while not being toxic to cells grown in traditional 2-D cultures. TNBCs comprise 10-20% of all breast cancers but are among the most aggressive and most lethal. Breast cancer remains the second leading cause of cancer death among women in the US and in Florida.

   Cancer cells grown as spheroids better mimic tumors and represent a more clinically relevant way to screen for potential new cancer treatments. The project has made significant progress. The first aim was to screen 3,000 samples from a library of enriched fractions obtained from marine organisms in two TNBC cell lines and has been completed. These cell lines are originated when patients have surgery to remove tumors and retain the characteristics of the tumor. The second specific aim is to purify the enriched fractions to isolate the compound responsible for the activity. To date, 11 marine natural compounds with the ability to induce apoptosis in triple negative breast cancer spheroids have been identified. Some of these compounds only show activity in cells grown as spheroids but not traditionally as a single layer. This suggests that these compounds act through unique modes of action and would have selectivity for tumors. The purification of other leads continues, presenting the opportunity that other compounds, perhaps more potent, will be identified.

The third specific aim of this study focuses on understanding how the compounds identified work. Two of the compounds were subjected to differential protein expression, a process that compares the proteins affected by treatment with a compound compared to a control. For one of these compounds, the results showed that the proteins upregulated or downregulated appear to correlate with better clinical prognosis of breast cancer patients. The results obtained allowed the formulation of a hypothesis of its possible mode of action, although it appears that this hypothesis is wrong. This could be the result of the uniqueness of the compound of only being active against spheroids. The results of the second compound have recently been received and are in the process of being analyzed in depth. Samples from cells treated with two other compounds are being prepared for differential protein analysis. Two compounds were also tested in combination with the known chemotherapeutic Taxol™ in the spheroid assay, and they exhibit synergy, which means that the results of the compounds together with the chemotherapy surpass the addition of the results seen with each treatment alone. These compounds will soon be tested to determine if they can stop the ability of spheroids to expand (invasion assay) to test in the lab if the compounds could stop metastasis, which is the spread of tumors to other organs.

There are no targeted therapies available for triple negative breast cancer. Therefore, the compounds identified through this grant have high potential to make a difference in patients with this aggressive form of breast cancer.

Follow on Funding: None at the time of reporting.

Collaborations: None at time of reporting.

Journals: Guzmán E.A., Pitts T.P., Winder P.L., Wright A.E. The Marine Natural Product Furospinulosin 1 Induces Apoptosis in MDA-MB-231 Triple Negative Breast Cancer Cell Spheroids, but Not in Cells Grown Traditionally with Longer Treatment.(2021). doi: 10.3390/md19050249 PMCID: PMC8145321

Patents: None at time of reporting.

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

Principal Investigator: Jennifer L. Steiner, PhD

Organization: Florida State University

Abstract: Colorectal cancer is among the most prevalent cancers and is the second leading cause of cancer related death. Frequent drinking of moderate to high levels of alcohol increases cancer risk. Cancer cachexia is present in ~50% of colon cancer patients and is characterized by the loss of skeletal muscle and fat mass which directly contributes to decreased muscle strength, quality of life, and treatment compliance and efficacy, as well as increased mortality. Lifestyle factors including alcohol intake, as well as treatments like chemotherapy, may worsen the development of cancer cachexia. The purpose of this project is to determine the impact of alcohol intake on cachexia development as well as the molecular changes incurred by either the

prior and/or continued intake of alcohol at tumor initiation. An additional aspect of this work is to investigate the functional impact alcohol may have on skeletal muscle performance in animals suffering from cancer cachexia as muscle weakness can greatly decrease quality of life. These research questions are currently being addressed using a mouse model of cancer cachexia in which colon cancer cells are implanted into the animal and cachexia develops over the subsequent weeks as the tumor grows. Two different models of alcohol consumption are currently under investigation to determine whether the cachectic effects differ if the patient stops drinking alcohol at the time, cancer is diagnosed versus continuing to drink post diagnosis. In the past year considerable progress was made once COVID related shutdowns (four to five months) ended and we could return to the lab. The research team has completed all of the animal experiments for Aim 1 and are nearing completion of the molecular analyses on the samples. The team hopes to publish these data within the year. Currently, the team is performing animal experiments for Aim 2 focused on the potential interaction of alcohol use and chemotherapy.

The experimental findings to date have shown that prior and continued alcohol intake increase the amount of body weight, fat mass and muscle mass lost following cancer, especially in male mice. Molecular analyses show that during cachexia development some proteins and genes responsible for maintaining muscle size are decreased while those contributing to the loss in muscle proteins are increased with alcohol which could be contributing the changes in muscle mass observed. Functional testing also shows greater losses in performance on coordination tests in alcohol treated cancer animals while analysis of muscle contraction strength measurements is ongoing. In many measures the group of animals that stopped consuming alcohol at the time of cancer initiation exhibited a very similar degree of change as that in the continued alcohol group, indicating alcohol use can have lasting harmful effects. Therefore, data thus far indicate that prior alcohol consumption, as well as continued alcohol consumption increases the severity of cancer cachexia as well as hastens the progression of the disease. Ultimately, this information will help inform Floridians how detrimental alcohol intake may be to their health and quality of life after getting a cancer diagnosis.

Follow on Funding: None at time of reporting.

Collaborations: None at time of reporting.

Journals: None at time of reporting.

Patents: None at time of reporting.

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

Principal Investigator: George S. Rust, MD, MPH

Organization: Florida State University

Abstract: Breast and colorectal cancers are two of the most screen able and treatable cancers, yet both still rank in the top five for cancer deaths. While death rates for each are declining in

the U.S., the racial gap in breast and colorectal cancer deaths has paradoxically widened. Minority and disadvantaged populations face barriers to obtaining cutting-edge screening, diagnosis, and treatment in different ways across diverse Florida communities. The purpose of this project is to help each community to understand where they can most strategically target local interventions to achieve the greatest impact on cancer outcomes. The research team has built predictive models, by analyzing data from cancer registries around the country merged with Medicare claims data to build models that predict cancer stage at diagnosis based on variables in our conceptual model. The Bayesian modeling approach was 22 times more accurate than a traditional statistical approach.The team has extended the relevance of models to predict cancer survival rates, and to distinguish variables that can't modify (such as a patients' sub-type of breast cancer) from variables that are potentially intervenable (improving cancer screening rates and decreasing biopsy delay or treatment delay). This will allow for "what-if" discussions with the community such as, "what-if we could get everyone to treatment without delay? How many lives could be saved?" Or, "should resources be spent on more mammography testing, or should the focus be on eliminating racial differences in access to cutting-edge treatments?"

The team is validating our models on Florida data and moving to county-level predictions using state cancer registries through the Florida Cancer Data System. The team is engaging and listening to community stakeholders through videoconference and telephone focus groups conducted with African American persons in five regions of Florida. The team is now seeking to understand how best to present the results visually, and how best to engage community members in discussions around "What will it take in the community to achieve equal outcomes in cancer?" Next steps involve data visualization and app development. The team relies on Florida Center for Interactive Media Development (FCIM) at Florida State University (FSU) for app / portal development in parallel with the model-building and community engagement. In the meantime, students are working on data visualization projects, to create and test the best interactive visualizations for presenting our decision tools for racial disparities in breast cancer across Florida.

Follow on Funding: None at time of reporting.

Collaborations: Faculty at the FSU College of Medicine and the FSU Department of Statistics including PhD and MD students in the team to provide learning opportunities and to train the next generation in science.

Journals: None at time of reporting.

Patents: None at time of reporting.

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

Principal Investigator: Gina M. DeNicola, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: Health Impact to Floridians

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

AIM1: Target NRF2-regulated metabolism for cancer therapy. The goal of this aim was to evaluate therapeutic approaches that target the metabolism of tumors with mutations in NRF2 and KEAP1, which are found in up to 30% of non-small cell lung cancers (NSCLC). Research project staff are evaluating two approaches, one to block the metabolism of a key nutrient, cysteine (approach #1), and one to exploit a NRF2-regulated enzyme, NQO1, to selectively kill mutant tumor cells

This approach has resulted in a publication describing a combination strategy to induce cytotoxicity in tumors based on NQO1-activatable compounds, in combination with inhibition of antioxidant enzymes that protect against the toxic effects of these compounds. To identify additional antioxidant enzymes that may be more tumor-selective, the research team has performed a CRISPR screen and identified two additional targets, which have now been validated. Further work has identified the mechanism of protection by these targets, which involves the mitochondrial antioxidant system. Additional CRISPR screening has identified new strategies to target NRF2 directly. Staff have also tested more potent NQO1-activatable compounds to attempt to overcome antioxidant protection. Despite improved potency in cell culture, limited efficacy in animals was observed. Progress on approach #2 has been limited by potential immunogenicity of our enzyme-based therapy and possible resistance of tumor cysteine pools to serum cysteine depletion. Testing of alternative approaches is ongoing.

AIM2: Relate NRF2/KEAP1 mutations and pathway activation with therapeutic response. The goal of this aim is to identify the appropriate patient cohorts to study the effect of KEAP1 and NRF2 mutation status on patients' response to chemotherapy, radiation therapy and immunotherapy. Patients were identified by leveraging Moffitt's enterprise wide data warehouse.

Chemotherapy response: Cohorts for the analysis of chemotherapy response have been assembled and study pathologists have completed review of H&E slides. The FFPE blocks with the best tumor representation have been identified. DNA extraction has been performed and the sequencing assay for NRF2 and KEAP1 has been tested. Sequencing of NRF2 and KEAP1 on the full cohort is now ongoing. The next steps are to correlate mutation status with chemotherapy response.

Patient analyses have been completed and research staff found that NRF2 activation was associated with regional nodal recurrence following radiation. These results suggest that patients with NRF2/KEAP1 mutations are more refractory to radiation, which should be considered in their treatment. The research staff now determining next steps. Two separate cohorts of NSCLC patients treated with PD-1 based immunotherapy with pre-treatment tissue

available were identified. Further data query to extract info on cancer characteristics, treatment history, and survival data is in progress.

Follow on Funding: None at time of reporting.

Collaborations: City of Hope, Department of Radiation Oncology, Duarte, California, Dr. Terrence Williams. Dr. Williams developed databases of patients with non-small cell lung cancer treated with radiation and chemoradiation at the Ohio State University and is an expert on DNA repair and DNA damage response.

Following the death of our collaborator David Boothman, who provided b-lapachone for our studies and was going to provide us the more potent compound IB-DNQ, we have established a collaboration with Dr. Paul Hergenrother, Department of Chemistry, University of Illinois at Urbana-Champaign to obtain IB-DNQ.

Journals: None at time of reporting.

Patents: None at time of reporting.

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

Principal Investigator: Nelli Bejanyan, MD

Organization: H. Lee Moffitt Cancer Center

Abstract: Higher γδ T cell counts in patients with relapsed or refractory acute myeloid leukemia is associated with better survival. However, γδ T cells are rare in the blood and functionally impaired in patients with malignancies. Promising results are reported on the treatment of various malignancies with in vivo expansion of autologous γδ T cells using zoledronic acid (zol) and IL-2. The research staff demonstrated that zol and IL-2, in combination with a novel genetically engineered K562 CD3/CD137L/CD28/IL15RA quadruplet artificial antigen presenting cell (aAPC), efficiently expand allogeneic donor-derived γδ T cells using a GMP-compliant protocol sufficient to achieve cell doses for future clinical use. This led to the U.S. Food & Drug Administration (FDA) approving the team's Investigational New Drug (IND) application to proceed with the "Phase 1/1b trial of donor γδ T-cell infusion for treatment of patients with acute myeloid leukemia at high risk of relapse after allogeneic hematopoietic stem cell transplantation." Using this methodology, the team achieved a 633-fold expansion of γδ T cells after day 10 of co-culture with aAPC, which exhibited central (47%) and effector (43%) memory phenotypes. Additionally, >90% of the expanded γδ T cells expressed Natural Killer Group 2D (NKG2D), while they have low cell surface expression of programmed cell death protein 1 (PD1) and LAG2 inhibitory checkpoint receptors. In vitro real-time cytotoxicity analysis showed that expanded γδ T cells were effective in killing target cells. The results demonstrate that large scale ex vivo expansion of donor-derived γδ T cells in a Guanosine monophosphate (GMP)-like setting can be achieved with the use of quadruplet aAPC and zol/IL-2 for clinical application. This work has now been submitted for publication to the Journal of Immunotherapy as of June

9, 2021. The clinical trial is now approved by IRB and we have now started screening patients for enrollment in our clinical trial.

In addition, γδ T cells and αβ T cells were used for chimeric antigen receptor (CAR0 transduction, which was performed on days one and two2, days two and three, and days three and four of expansion to identify the optimal CAR transduction timing. These experiments were repeated three times using three different donors and we identified that the optimal CAR transduction timing are the days one and three. Additional preclinical studies comparing the cytotoxicity of γδ CAR T cells and αβ CAR T cells are in progress.

Follow on Funding: None at time of reporting.

Collaborations: CareDx pharma confirmed their interest to collborate with us on this project and will provide the funding to support this research.

Journals: None at the time of reporting.

Patents: None at time of reporting.

6. **Grant #:** 9BC09 Development of Novel TAF1 Inhibitors as Cancer Therapeutics

Principal Investigator: Ernst Schonbrunn, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: The bromodomain-containing protein TATA-Box Binding Protein Associated Factor (TAF1) is an essential subunit of the general transcription machinery. Although deregulation of gene transcription and evolving plasticity are the underlying cause of drug resistance in cancer, TAF1 is a potential target for the development of drugs aimed at uncontrolled gene transcription. To date, only few bromodomain inhibitors of TAF1 have been developed, but biological effects have not been reported, and no TAF1 inhibitor has reached the clinic. The present knowledge suggests that TAF1 is a promising yet underexplored target for the development of small molecule inhibitors directed at the transcription machinery of cancer cells through an epigenetic mechanism of action.

Recently, the research team discovered that a clinical inhibitor of the protein kinase ataxia telangiectasia and Rad3 related (ATR) also selectively inhibits the second bromodomain of TAF1. This is the first identified kinase inhibitor that targets bromodomains outside the bromodomain and extraterminal domain (BET) family. The team determined high resolution co-crystal structures of TAF1 liganded with this inhibitor and close analogues, the knowledge of which provides a new structural framework for the rational design of inhibitors with high potency and selectivity for TAF1 and the ability to concurrently inhibit ATR or other PI3K-related kinases. Preliminary studies in lung and colon cancer cell lines established that TAF1 inhibitors activate p53 and DNA damage response and cell death.

The central hypothesis of this proposal is that TAF1 inhibition by small molecules is a viable strategy to alter the transcription machinery of cancer cells. The scientific premise is the

knowledge gap about the efficacy of chemical inhibition of TAF1 alone and in combination with ATR in cancer. The objectives are the development and in-depth characterization of novel inhibitors that potently inhibit TAF1 and ATR. This proposal integrates research components from structural biology, cancer biology and medicinal chemistry for the development of dual TAF1-ATR inhibitors as cancer drugs.

Progress during the past year involves cell biological, chemical, and structural studies. New analogues of AZD6738 were synthesized and characterized for inhibitory potential against TAF1 and ATR, and a patent application for these new inhibitors has been filed. The team also synthesized a previously reported potent TAF1 inhibitor (GNE371), and a new series of compounds was designed and synthesized by merging the chemical scaffolds of AZD6738 and GNE371. New high-resolution cocrystal structures of the TAF1 tandem bromodomain were determined for analogues of AZD6738, GNE371 and merged scaffold compounds. The crystal structure information guided the design of compounds with increased binding potential and the development of heterobifunctional proteolysis-targeting chimera to selectively degrade TAF1.

To determine whether p53 is a suitable biomarker for the functional analysis of TAF1 inhibitors, cancer cell lines expressing wildtype p53, p53-null cells as well as p53 knockdown by siRNA were utilized. Of all inhibitors tested, only the previously reported compound BAY299 significantly induced p53 activation. However, the data suggest that p53 activation by BAY299 is caused by an unknown off-target mechanism independent of TAF1 bromodomain inhibition. It appears that p53 is not a biomarker for TAF1 bromodomain inhibition, and further mechanistic studies are underway.

Follow on Funding: None at time of reporting.

Collaborations: None at time of reporting.

Journals: None at time of reporting.

Patents: Ernst Schonbrunn, Justin Lopchuk, Rezaul Karim, TAF1 inhibitors, International patent application PCT/US21/23419, 03/22/2021, Moffitt Cancer Center

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

Principal Investigator: Anthony J. Capobianco, PhD

Organization: University of Miami

Abstract: Aberrant activation of Wnt/β-catenin signaling is observed in various types of cancers particularly in colorectal cancer (CRC) where it promotes uncontrolled proliferation, invasiveness of cancer cells and supports cancer stem cell maintenance and resistance to chemotherapy. Presently, there are no small molecule inhibitors in the clinic that target the nuclear β-catenin transcriptional complex and selectively inhibit the Wnt/β-catenin pathway. The aim of this study is to develop novel potent drug-like small molecule inhibitors of the Wnt/β-catenin transcriptional activation complex. Previously, three scaffolds, BC-57, BC-14 and BC-45

that target the BCL9/β-catenin interface were identified for further development. The research staff has prepared or acquired ~100 analogs for the BC-57 and used structure-activity relationship (SAR) study and medicinal chemistry to progress in validation of lead scaffold SSTB-178 and its derivatives. To further characterize the biological effects of the analogs on growth of colorectal cancer cells, cell viability assay (CellTiterGlo), and colony formation assay have been carried out. Non-specific toxicity was evaluated by observing the effect of these analogs on growth of β-catenin-independent cell line RKO as well 293 cells. The lead scaffold SSTB-178 showed improved potency as evaluated by its inhibitory concentration (IC50) values.

Mouse xenografts were used to evaluate the effect of lead candidate SSTB-178 on tumor growth in vivo. HCT-116 cell line-based xenograft were treated with 40 mg/kg intraperitoneal (IP) of compound daily. It was observed that the growth of the SSTB-178 treated tumor and the size of the tumors were reduced compared to the control group of mice. Further assessment of the effect of treatment on tumors was done by analysis of biomarkers. The tumors were harvested, cells from excised tumors and were subjected to RT-qPCR analysis. Expression of Wnt target genes were reduced in the SSTB-178 treated tumor derived cells. This indicates that the lead compound SSTB-178 targets Wnt pathway specifically in vivo.

Over the past decades, small molecules which inhibit components of Wnt pathway have been identified. These include molecules which target Wnt secretion (the porcupine inhibitor IWP-2 or the Wnt receptor complex (Dishevelled/Frizzled interaction inhibitors NSC668036 and FJ9), or modulators of beta-catenin destruction complex (tankyrase inhibitors XAV939 and JW55) as well as agents that disrupt the transcription complex (beta- catenin/ CBP interaction inhibitor ICG-001, beta-catenin/TCF interaction inhibitors iCRT3 and CWP232228. The inhibition brought on by these molecules potentially causes toxicities and cross-regulatory effects and are ineffective in human colorectal cancer, which frequently have APC and Axin inactivation mutations or β-catenin activation mutations. The research team reasoned that our efforts to develop new Wnt inhibitors directed to the downstream beta-catenin–BCL9 transcription complexes would lead to a novel therapeutic to treat Wnt-dependent colorectal cancer.

Follow on Funding: None at time of reporting.

Collaborations: None at time of reporting.

Journals: None at time of reporting.

Patents: None at time of reporting.

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

Principal Investigator: Kerry L. Burnstein, PhD

Organization: University of Miami

Abstract: In Florida in 2021, 19,950 men will be diagnosed with prostate cancer (PC) and 2,850 will die of this malignancy, per American Cancer Society estimates. In advanced PC, tumors

often develop “resistance” to drugs, leading to incurable cancer growth. Tumors become resistant in ways that vary between patients. Thus, treating aggressive and drug resistant PC requires tailoring therapies specifically to features of individual patients’ tumors. Fortunately, huge amounts of molecular, genetic and clinical information (“big data”) on PC from a broad variety of patients exist. Project staff are exploiting these data to identify and prioritize new and existing drugs and drug combinations to treat PC more effectively. Significant progress was made in two major areas: the computational framework for evaluating new therapeutics was greatly expanded based on PC tumor data; andnew test compounds (identified by computationally screening millions of compounds for decisive properties of known drugs) were tested on PC cell lines (analyzed for genetic characteristics that must be targeted by new therapeutics).

Data from 501 PC patient tumors and 52 non-cancer prostate samples was acquired and processed using publicly available information. PC-specific gene “signatures” were identified for computational screening against the constellation of drugs and drug combinations. This method produced “modules” that identify classes of genes or proteins that are over-represented in large numbers of patients and are associated with disease state and patient condition. To generate usable information from the vast field of data types, an integrative database was developed to predict and prioritize effective PC therapeutics.

For “real world” compound testing, a refined assay was developed that combines live cell imaging (providing estimates of cell growth and evidence of cancer-like changes) and endpoint assays (determining drug-induced cellular toxicity). A liquid handling robot (purchased with university funds not derived from this Bankhead-Coley award) was employed to increase experimental throughput and to fortify inter-experimental robustness.

To date, a total of 33 compounds have been tested against a panel of cell lines (from PC, advanced PC, and non-cancer prostate tissue). All cell lines were thoroughly characterized by “RNAseq,” a method that reveals the “deep” genetic characteristics of (and differences between) these cell lines. A significant proportion of these compounds have been vetted in human clinical trials and may be combined with other drugs in novel ways leading to synergism, where clinical responses from combinations are greater than the sum of the individual drugs. Two main groups of compounds were identified: (1) compounds that reduced viability across all evaluated PC cell lines independent of biological and genetic differences between cell lines; and (2) compounds that specifically inhibited certain PC cell lines but not others. Experimental results from tested compounds will be fed back into the computational screening engine to sharpen the resolution of its predictions. High-performing compounds will be tested in combinations to detect synergism.

A large amount of PC data has already been curated and organized and will be made publicly available as a permanent resource to benefit the PC research community and to maximize the project’s impact.

Follow on Funding: None at time of reporting.

Collaborations: Stephan Schürer, PhD, University of Miami (collaborator) leads the computational discovery aspects of the project.

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

Rimpi Khurana, PhD, University of Miami (post-doc) performs RNAseq processing and analytics including gene set enrichment and network analysis.

Beronica Ocasio, University of Miami (graduate student) performs development and implementation of multi-parametric prioritization and search algorithms, under the guidance of Dr. Stathias, to identify the best drugs and drug combinations to test in prostate cancer cells and PDX models.

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

Nahuel Peinetti, PhD, University of Miami (post-doc) assists Dr. Martinez with cell-based studies and conducts tumor analysis (examining xenograft tumor growth and ex vivo analysis of tumors).

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

Journals: None at time of reporting.

Patents: None at time of reporting.

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

Principal Investigator: Hong Yuan (Rays) Jiang, PhD

Organization: University of South Florida

Abstract: Cancer cells rewire their metabolic pathways to fuel rigorous cell growth and replication. This is a report the research staff's progress in this BHC project, on a novel pan-cancer metabolic reprogramming mechanism, called 'heme-overdrive', that operates in hundreds of cell lines derived from diverse tissues, and in primary tumors from patient biopsies.

To demonstrate the significance of the project, the team has made progress in showing that heme overdrive is universal and unique to a wide range of cancer types and is absent in normal differentiated tissues, as evidenced by whole genome clustered regularly interspaced short palindromic repeats knock out (CRISPR KO) analysis, single-cell RNAseq studies and biochemical validations.

To show the therapeutic potential of exploiting the findings, the team has shown that heme overdrive is biochemically and genetically distinct from other pathways, with canonical features including enhanced heme flux, imbalanced heme biosynthesis and accumulation of biochemical intermediates (e.g., porphyrins). These findings illustrate that therapeutic methods can be developed against cancers with minimum damages to the normal tissues.

Mechanistically, imbalanced heme biosynthesis is exploited by cancer cells to ramp up production of heme-related intermediates; and heme can act as a master epigenetic regulator for oncogenic signaling to directly active RAS and angiogenesis processes. Furthermore, heme-overdrive is likely enabled by cancer microenvironments that supply flux of substrates, as shown by single-cell RNAseq of primary tumors. At this point of the project, the team discovered that heme-overdrive is a common biochemical reprogramming feature employed by cancers, akin to the 'Warburg effect' of altered onco glucose metabolisms; and decoding the biochemical underpinnings may allow for the identification of novel cancer metabolic vulnerabilities which can be used for novel therapeutic strategies for cancer.

For the experimental platform and future expansion, the team has achieved the establishments of three major experimental systems, i.e. 1) high-throughput and high-resolution drug essays for 'bait-and-kill', based on the principle of cancer heme overdrive. 2) single-cell measurements of transcriptome, metabolites and Reactive Oxygen Species (ROS). 3) validation of heme-overdrive with a set of CRISPR mutants.

The research team has operated the labs and training under restrictions imposed by COVID-19. The team has adapted the working schedule and managed to train students and disseminate the team's research results. The team has trained graduate students and junior researchers in cutting-edge technologies and life science lab operational skills. The team has established a collaborative network with four partners in three institutions. The team has presented the work at various invited talks in international and national meetings with adequate acknowledgment to the Forida state funding.

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

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

Dr. Ferreira (USF) has close collaborations with Dr. Jiang, and this work would not be possible without their weekly joint lab meetings and very frequent discussions.

Dr. Reuther's (Moffitt Cancer Center) interest in cell signaling pathways, particularly in the field of myeloid leukemia.

Dr. Sebti (VCU) is a recognized cancer researcher that holds the NCI Outstanding Research award.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Bankhead-Coley Cancer Biomedical Research Program
Appendix D
Fiscal Year 2020-2021 Active Grants
Funding Fiscal Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
8BC05	University of Central Florida	Otto Phanstiel, PhD	$815,283	09/31/2021	Yes	Yes	Yes

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

Principal Investigator: Otto Phanstiel, PhD

Organization: University of Central Florida

Abstract: The purpose of the grant was to discover new polyamine transport inhibitors (PTIs) and use these to better understand the role of polyamine metabolism in pancreatic cancers. The goal was to develop new therapeutic candidates which deplete polyamine supplies in tumors and activate the immune response as a way to treat pancreatic cancers. Along this discovery path, compounds were found with very unique properties and pivoted to pursue these exciting molecules which allow one to reprogram tumors into an anti-tumor phenotype.

Pancreatic cancer is one of the world's most deadly cancers with a five-year survival rate of <9%. New therapies are desperately needed as the current therapies only provide a six to eleven-month life extension. Immunotherapies have essentially failed in pancreatic cancers. These tumors have high levels of polyamines and the team's hypothesis is that they use their polyamines to establish immune privilege. The polyamine spermine is immune suppressive and pancreatic tumors have high expression of the polyamine transporter (ATP13A3). The team developed therapies that result in polyamine depletion and take away the polyamine immune shield established by the tumors. A success here should enable immunotherapies to work in pancreatic cancers.

The research staff has synthesized and screened a large number of compounds in vitro and discovered that the lead compounds act not by blocking polyamine import as originally thought but by inhibiting the far upstream binding protein 1 (FUBP1). FUBP1 interacts with single-stranded DNA upstream of specific genes involved in tumor survival such as c-myc and p21. The research team has shown that the lead compounds reprogram the cells to an anti-cancer phenotype, where they decrease c-myc and increase p21 expression. This genetic reprogramming by a small molecule is novel and provides an exciting new way to treat cancers by inhibiting the transcription factor FUBP1, which regulates the expression pattern of key genes that tumors rely on for survival. The research team's top performing FUBP1 inhibitor, UCF699, outperformed the best known FUBP1 inhibitor (the Hauck inhibitor) in a head to head

comparison for their ability to decrease c-myc and increase p21 both at the mRNA and protein levels

The research team's success has led to a new patent claiming the intellectual property for UCF and is under review by the US Patent Office. Once awarded, the patent can be licensed to pharmaceutical companies interested in marketing this discovery. The research team anticipates that this new approach will significantly impact the healthspan of pancreatic cancer patients by reprogramming specific genes in the tumor so that the patient's own immune system can better attack the tumor.

Follow on Funding: None at the time of reporting.

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

Journals: Vandana Sekhar, Thomas Andl, and Otto Phanstiel IV. ATP13A3 facilitates polyamine transport in human pancreatic cancer cells. Cellular Oncology, (2021).

Patents: None at the time of reporting.

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

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
7BC03	University of Miami	Emmanuel Thomas, MD	$1,866,436	4/30/2021	No	No	No
7BC04	H. Lee Moffitt Cancer Center	Clement K. Gwede, PhD, MPH, RN, FAAN	$828,125	3/31/2021	No	Yes	Yes

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

   Principal Investigator: Emmanuel Thomas, MD

   Organization: University of Miami

   Abstract: The research team's greatest achievement for the last year was having the state of Florida being recognized for its efforts to eliminate Hepatitis C (HCV) (https://www.hhs.gov/hepatitis/get-involved/hepatitis-elimination/index.html). Furthermore, the team has created the HCV-free Florida website (www.HCVFreeFL.com) and an associated Twitter account to support the HCV elimination efforts in Florida. Through this funding, the team is also assessing the impact of COVID-19, and associated variants, on liver disease progression to hepatocellular carcinoma. Overall, the study team is very excited about this project and are confident that the goals will be achieved. The team has collected clinical information and now have a comprehensive database for 2,080 patients with liver disease that are at increased risk of developing hepatocellular carcinoma (HCC). As described in Aim1 of the grant, the team has completed the cross-sectional analysis that will be carried out now in 2,080 patients to identify novel clinical covariates that may drive liver disease progression. The goal is to identify covariates that may drive hepatocarcinogenesis in order to identify Floridians who are at risk earlier so that interventions can be employed. Emphasis in on future work will be focused on Floridians with highest and intermediate risk of developing HCC and trying to generate a new risk calculator that incorporates Fibroscan. Toward the initial efforts to develop a liver cancer risk calculator that utilizes race/ethnicity, the team has begun to develop new non-invasive prediction models for fibrosis and cirrhosis. Since cirrhosis is the most powerful predictor for the risk of developing HCC and because liver biopsies are being utilized less by the clinical community, the team believes these efforts will lay the foundation for future work. Using multivariable statistical modeling, the team was able to accurately predict cirrhosis (Metavir F4 fibrosis stage) utilizing noninvasive clinical markers and are now mapping liver disease based on the Zip Code based data. Importantly, the team has submitted follow on grants to the National Institutes of Health (NIH), Department of Defense and a Bankhead Infrastructure grant that will take our work and expand it to the rest of Florida by leveraging the OneFlorida consortium based at the University of Florida. Furthermore, since starting this Bankhead-Coley Grant, the Principal Investigator (PI) has been awarded a five-year, renewable grant from the National Institutes of Health (NIH) for $1.9 million. This NIH funded study is focused on understanding inflammatory mechanism that lead to chronic viral infections in the liver through basic science laboratory studies. The grant is a nice complement to this clinical study, supported by the Florida Department of Health, and the funding from this grant has increased

since the team subsequently received a minority supplement to support a graduate student. In addition, the PI has been awarded a new $300,000 grant from Gilead Sciences to screen for HCV and HIV in the University of Miami Emergency Department. Furthermore, the team recently established the Florida HCV-HCC/Liver Cancer Consortium with Moffitt Cancer Center, University of Florida and Jacksonville Mayo Clinic through four previous meeting.

Follow on Funding: None at time of reporting.

Collaborations: This project is being performed at the University of Miami Miller School of Medicine. Furthermore, we recently formed the Florida HCV-HCC/Liver Cancer Consortium with Tampa Moffitt Cancer Center (Dr. Anna Giuliano-Center for Infectious Cancers), University of Florida Gainesville (Drs. David Nelson-Hepatology and Betsy Shenkman-Medicine) and Jacksonville Mayo Clinic (Dr. Tushar Patel-Transplant Hepatology)

This project is currently providing training to four University of Miami Graduate students: Dennis McDuffie (3rd year graduate student-PhD program), Jasmine Edwards (3rd year graduate student-PhD program), Owen Willis (2nd year graduate student-PhD program) and Alejandro Badilla (2nd year graduate student-PhD program) and three University of Miami undergraduate students (David Barr, Robert DiCaprio and Danae Lally).

Journals: None at time of reporting.

Patents: None at time of reporting.

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

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

Organization: H. Lee Moffitt Cancer Center

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

Specific Aim #1: To test whether C-CARES Plus (education + FIT + personalized components) compared with C- CARES (education + FIT) improves long-term FIT screening adherence among 328 individuals, 50-75 years of age, who are not up-to-date with CRC screening.

The study is closed to accrual as targeted enrollment was achieved (last case enrolled: 11/26/2019). The cumulative accrual of 328/328 represents 100% of projected accrual. The cumulative study outcomes were summarized in the annual progress report (e.g., FIT uptake and follow up of abnormal results) submitted 1/28/2020. Participants in both study arms continue in follow-up for certain procedures (e.g., surveys) including FIT kit return.

A total of 61 (46 from baseline, 14 from 12-month follow up, 1 from 24-month follow-up) abnormal FIT results have occurred since initiation of the study. Overall, twenty-four of the participants have completed their colonoscopy screenings. Fifteen participants are pending and being tracked for colonoscopy completion either by the Moffitt team or navigated through the clinics' usual care practice. Another 22 participants are no longer being tracked, are considered resolved and closed out per clinic procedure/protocol due to the following: unable to reach after

multiple contacts [n=3], refused colonoscopy [n=6] or per clinic procedure, uncompleted colonoscopy despite multiple interactions with patients [n=13]. CCARES-Plus arm coaching for those who did not return FIT after 90 days of receiving FIT, Baseline coaching completed in April 2020. A cumulative total of 36 participants completed baseline coaching for the study duration. 12-month coaching is ongoing, no participant required coaching this quarter. A cumulative total of 48 participants have completed coaching at the 12-month assessment interval.

For CCARES-Plus arm 12-month follow-up and booster education the 12-month follow-ups surveys completed January 2021, a cumulative total of 85 participants completed the follow-up survey. Booster education distribution completed February 2021, a cumulative total of 134 booster educations (1-page educational leaflet) were sent out. For CCARES arm 12-month Contact Cards distribution was completed December 2020, a total of 143 participants were mailed a contact card (generic CRC message) via post-card at 12-month interval per protocol. For both CCARES and CCARES-Plus arm 24-month follow-up surveys began April 2020 and are ongoing, a total of 74 participants have completed the survey.

As far as impact to Floridians, the produced CRC English/Spanish educational DVD and photo novella booklets developed as part of the study are currently being used as the basis of the intervention and access to screening is provided via the FIT test for study participants and in the clinics overall per usual care practices. FIT screening rate of 69% at baseline for the study matches the State's general population average and far exceeds the rates of approximately 44% (2018 HRSA data) seen in Florida's Federally Qualified Health Clinics.

Follow on Funding: NIH, Clement K. Gwede, PhD and Cathy D. Meade, PhD, $3,145,317

Collaborations: None at the time of reporting.

Journals: Christy, SM, Sutton, SK, Abdulla, R, Boxtha, C, Gonzalez, Paola, Cousin, L, Ewing, A, Montoya, S, Lopez, Beehler, T, Sanchez, J, Carvajal, R, Meade, CD, Gwede, CK. A Multilevel, Low Literacy Dual Language Intervention to Promote Colorectal Cancer Screening in Community Clinics: A Randomized Controlled Trial (2021).

Patents: None at time of reporting.

Bankhead-Coley Cancer Biomedical Research Program
Appendix F
Fiscal Year 2020-2021 Active Grants
Funding Fiscal Year 2015-2016

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
6BC09	University of Florida	Walter G. O'Dell, PhD	$1,445,737	6/30/2021	Yes	Yes	Yes

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

Principal Investigator: Walter G. O'Dell, PhD

Organization: University of Florida

Abstract: This project focused on better documenting and understanding the biological mechanisms and factors that influence radiation damage to the lung in breast cancer patients. Although radiation therapy (RT) is overall beneficial to breast cancer patients, around 14% will require medical intervention to treat lung toxicity following treatment. In particular, the research team was interested in quantifying the reduction in lung toxicity afforded through the use of proton therapy versus standard X-ray based RT. The lab has developed tools to quantify both tissue and vascular damage from CT scans of the chest, and the team will correlate these changes with clinical findings of lung distress and patient survival, with pulmonary function (breathing) tests, blood markers of tissue toxicity, and quality of life surveys. Forty-one breast cancer patients were enrolled with 38 providing complete or near-complete data. For each subject the team acquired baseline and follow-up CT scans, blood draws, pulmonary function tests, and quality of life surveys. The ultimate goal is to develop mathematical models of the biological process of lung tissue and lung blood vessel damage that includes the contributions of patient-specific factors such as age, gender and smoking history, and also considers the type of radiation treatment and the effects of chemotherapy.

Documenting the effects of radiation on blood vessels in the lung requires extraction of the vessel trees from the CT scans and accurate assessment of vessel branch size. The team has patented a method to mathematically model the appearance of a simulated vessel branch on a CT scan and use this to optimize the radius and trajectory of each branch in a vascular tree. This method was validated using three-dimensional print-out of a real lung arterial tree that was extracted from the chest CT scan of human volunteer. Student researchers manually measured the radius of 69 branches in this 3D printed tree. The team then endeavored to calibrate the vessel counts for different pixel sizes and reconstruction filters. The team is currently analyzing the last few chest CT scans. An initial analysis showed that smoking history greatly impacts the number of blood vessels in the lung and their changes after radiation. Meanwhile, the team has completed analysis of the serial blood samples for all patients, focusing on 13 key markers of tissue injury. Statistical modeling showed that the 13 markers can be reduced to four groups that independently relate to patient and treatment factors. Presumably, each group represents a

unique response pathway where multiple markers are associated. A preliminary analysis showed that lung tissue reaction is most strongly associated with three of the blood marker groups, patient age, smoking history, and volume of lung radiated. Independent of lung response, two of the blood markers are strongly associated with patient size and indicated by the body mass index (BMI). Elevate BMI results in higher concentration of these markers after chemotherapy, and greater change after radiation treatment. The team's task in the coming months is to finalize the vessel and tissue analysis and perform statistical modeling to pull together the variety of patient and treatment factors into a cohesive predictive model of a patient's risk for severe toxicity.

Follow on Funding: None at time of reporting.

Collaborations: This project was a collaborative effort of the University of Florida Proton Therapy Institute and the UF Gainesville departments of Radiation Oncology, Medical Oncology, Radiology, Biostatistics, and Biomedical Engineering.

Journals: Begosh-Mayne D, Kumar SS, Toffel S, Okunieff P, O'Dell W. The dose-response characteristics of four NTCP models: using a novel CT-based radiomic method to quantify radiation-induced lung density changes. in Nature: Scientific Reports. (2020) doi:10.1038/s41598-020-67499-0. PMCID: PMC7324586

Saini A, Siva Kumar S, O'Dell W. Measuring lung vessel tree growth during development in pediatric patients. In UF Journal of Undergraduate Research. (2020) doi.org10.32473/ufjur.v21i2.108563

Patents: None at time of reporting.

Bankhead-Coley Cancer Biomedical Research Program
Appendix G
Fiscal Year 2020-2021 Completed Grants
Funding Fiscal Year 2019-2020

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
20B02	Mayo Clinic Jacksonville	Derek C. Radisky, PhD	$99,999	10/31/2020	No	No	Yes

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

Principal Investigator: Derek C. Radisky, PhD

Organization: Mayo Clinic Jacksonville

Abstract: The purpose of this project was to support the application for a NIH R01 application. The work performed using this bridge project funding was largely in support of Aim 2, which was seen by the study section as most in need of supplementary experiments. The scope of work did not overlap with the proposed experiments in the R01 application but served to strengthen the research plan. The research team had previously performed array-based profiling of a set of patients which had been diagnosed with benign breast disease (BBD) for which had been sequential, metachronous tissue biopsies. The team identified genes which were differentially expressed between women who showed LI progression or LI stasis, and selected 78 genes for validation. The team performed NanoString-based validation of these genes, and selected 23 genes which showed differences in gene expression between the LI progression and LI stasis groups. Bioinformatic analysis suggested that these genes might be organized into three coregulated groups. Several genes were identified from each of these groups for subsequent evaluation using immunohistochemistry-based approaches, which is currently being performed. While distinct from the work proposed in the R01 application, the results obtained here strongly support the feasibility of that research plan, and the R01 application was approved for funding. The team will continue with evaluation of the novel biomarkers identified through the work performed for the bridge project in parallel with the proposed work in the newly-funded R01 application.

Follow on Funding: NIH/NCI, Involution-Based Biomarkers of Breast Cancer Risk, Derek Radisky, $554,827

Collaborations: None at time of reporting.

Journals: None at time of reporting.

Patents: None at time of reporting.

Bankhead-Coley Cancer Biomedical Research Program
Appendix H
Fiscal Year 2020-2021 Completed Grants
Funding Fiscal Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
8BC01	Mayo Clinic Jacksonville	John Copland, PhD	$815,283	4/30/2021	No	No	No
8BC03	H. Lee Moffitt Cancer Center	Keiran S.M. Smalley, PhD	$815,283	3/31/2021	No	Yes	Yes
8BC04	H. Lee Moffitt Cancer Center	Robert J Gillies, PhD	$815,283	3/31/2021	No	Yes	Yes
8BC06	University of Miami	Xiangxi Mike Xu, PhD	$815,283	3/31/2021	No	Yes	No
8BC07	University of Miami	Ashok K Saluja, PhD	$815,282	3/31/2021	No	No	No
8BC09	University of Miami	Eric D Wieder, PhD	$1,358,805	3/31/2021	No	Yes	No
8BC10	University of Miami	Shanta Dhar, PhD	$815,283	3/31/2021	No	Yes	Yes

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

   Principal Investigator: John Copland, PhD

   Organization: Mayo Clinic Jacksonville

   Abstract: A key mediator of Fatty Acid (FA) biosynthesis, stearoyl CoA desaturase one (SCD1) is rate-limiting in the conversion of saturated fatty acids (SFA) to monounsaturated fatty acids (MUFAs) which are preferentially transformed into triglycerides for storage or phospholipids for membrane formation. SCD1 is overexpressed in most aggressive cancers. The research team published cell culture and animal data demonstrated endoplasmic reticulum (ER) stress induced cell death as a mechanism of action for antitumor activity. The team developed four novel SCD1 inhibitors. Two lead inhibitors block SCD1 enzymatic activity with EC50s of 1.9 (SSI-4) and 29 nM (SSI-2). SSI-4 induced apoptotic cell death via ER stress across a wide range of cancer histotypes, is well tolerated and possesses single agent antitumor activity. The team showed that inhibition of SCD1 increases the immunogenicity of poorly immunogenic tumors. Enhanced immune activation is due to upregulated ER stress. SCD1 inhibition increased recruitment and activation of immune cells in vivo, which when combined with PD-1 blockade resulted in potent and durable anti-tumor T cell responses in breast cancer mouse models. Thus, aberrant de novo lipogenesis is linked to attenuated tumor immunogenicity and SCD1 inhibitors are immuno-sensitizing agents. The team will develop SSI-4 combination therapy with anti-PD-L1 immune checkpoint inhibitors using mouse models of breast and colon cancers and melanoma leading to another patent filing to protect and enhance commercialization potential via successful clinical trials. The team has proposed three aims to reach these goals: demonstrate antitumor synergy and survival benefit of SSI-4 plus anti-PD-L1 antibody in breast and colon cancers and melanoma, examine mechanisms of action whereby SSI-4 sensitizes tumors to

checkpoint inhibitors, and write a clinical trial. In summary, the team is developing novel SCD1 inhibitors which are currently not in development for the treatment of cancer. The team predicts that these inhibitors will find broad applicability and benefit patient survival. The team has experiments in progress with BioXcell anti-PD1 or anti-PD-L1 antibodies combined with SSI-4 testing anti-tumor synergy against mouse triple negative breast cancer cells implanted into mice mammary fat pad. Accomplishments in the 4th Quarter 2020 include continuing to move forward with the novel concept from the 3rd Quarter 2020 report where the team discovered that double negative (DN) T-cells (CD4-/CD8-/CD3+) are enriched in the tumor microenvironment when mice are treated with SSI-4. Progress continues to be made in writing and detailing the clinical trial strategy. The flow chart below indicates planned clinical trials. The has identified multiple indications for SSI-4 in combination with immune therapy that include two subtypes of breast cancer – triple negative and HER2 positive.

Follow on Funding: None at time of reporting.

Collaborations: None at time of reporting.

Journals: None at time of reporting.

Patents: None at time of reporting.

2. **Grant #:** 8BC03-A1 Defining and therapeutically targeting HDAC8-driven reprogramming in melanoma brain metastasis development

Principal Investigator: Keiran S.M. Smalley, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: Central Nervous System (CNS) involvement is clinically evident in ~30% of melanoma patients (as high as 75% at autopsy), and the brain is often the major site of disease progression; even when extracranial disease is well controlled. The core scientific premise of this work rests upon the discovery of an epigenetic state regulated through histone deacetylase (HDAC)-8 that reprogrammed melanoma cells and led ultimately to the establishment of metastases (including the brain) in vivo. The research team has hypothesized that HDAC8 regulates a stress-response transcriptional program that increases the resilience of melanoma cells, increasing the interactions with the vasculature, allowing the cells to evade immune detection and permitting those cells to form brain metastases. Over the lifetime of the grant, significant progress has been made in all three specific aims. The goal is to complete the two ongoing studies on the mouse model of HDAC8 in melanoma development/metastasis and our epigenetic analysis of HDAC8 function. Both studies are expected to be completed and submitted later this year. The team has already developed a follow-on R01 grant that was reviewed favorably at NCI (21st percentile) that focuses upon the role of HDAC8 in stress-dependent transcriptional reprogramming and its role in immune escape and metastasis. This grant will be resubmitted in November of 2021. A second R21 grant was developed and submitted in February of 2021 and is scheduled to be reviewed in June of 2021. This application focuses upon our observation that UV radiation can activate HDAC8 in melanocytes, leading to

the immune escape associated with melanoma development. The goal of this R21 will be to evaluate whether topical HDAC8 inhibition could be used as a melanoma prevention strategy.

Follow on Funding: Melanoma Research Foundation, Micheal Emmons, $100,000

Collaborations: This project is a collaboration between the lab of Dr. Smalley (Moffitt) and Dr. Licht (University of Florida).

Dr. Michael Emmons (Smalley lab) spent a week in the Licht lab in Gainesville learning ATAC-Seq and CRISPR screening in February of 2019.

Journals: HDAC8 regulates a stress response pathway in melanoma that mediates escape from BRAF inhibitor therapy. Michael F. Emmons, Fernanda Faião-Flores, Ritin Sharma, Ram Thapa, Jane L. Messina, Juergen C. Becker, Edward Seto, Vernon K. Sondak, John M. Koomen, Y. Ann Chen, Eric K. Lau, Lixin Wan, Jonathan D. Licht, and Keiran S.M. Smalley. Cancer Research. (2021)

Noncanonical EphA2 Signaling Is a Driver of Tumor-Endothelial Cell Interactions and Metastatic Dissemination in BRAF Inhibitor Resistant Melanoma. Chao Zhang, Inna Smalley, Michael F Emmons, Ritin Sharma, Victoria Izumi, Jane Messina, John M Koomen, Elena B Pasquale, Peter A Forsyth, Keiran S M Smalley. Journal of Investigative Dermatology. (2021)

Melanoma brain metastases: Biological basis and novel therapeutic strategies. Phadke, M., Ozgun, M., Eroglu, Z., Smalley, K.S.M. Experimental Dermatology. (2021).

Patents: None at time of reporting.

3. **Grant #:** 8BC04-A1 Targeting the Lipogenic Phenotype Induced by Extracellular Acidosis in Breast Cancer

Principal Investigator: Robert J Gillies, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: The extracellular pH of tumors is unequivocally acidic due to altered metabolism of cancer cells and insufficient perfusion. Studies from our lab have shown that microenvironmental acidity promotes local invasion, metastasis and resistance to immune surveillance. It is axiomatic that cancer cells need to adapt to acidic environment to survive and thrive. Acid adaptation involves three interrelated processes: chronic autophagy, redistribution of lysosomes to the plasma membrane, and dramatic increase in accumulation of cytoplasmic lipid droplets (adiposomes). The acid signal is perceived at the cell surface through acid sensors like T-cell death-associated gene 8 (TDAG8) and Ovarian Cancer G (OGR1). We depleted these G protein coupled receptors (GPCRs) using Clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) and observed that OGR1 (but not TDAG8) depletion inhibited acid-induced adiposome accumulation in Michigan Cancer Foundation 7 (MCF7) and T47D cells. To better characterize this system and identify additional targets, we will investigate

signaling downstream of OGR1 and functionally characterize OGR1 knockout cells and unravel the signaling cascade. We will assess if OGR1 depletion affects tumor growth and metastasis in immune competent xenograft models. Paradoxically, we and others have observed concomitant increases in fatty acid synthesis and βeta-oxidation under acidosis. Inhibition of either pathway was significantly toxic to cells under acidic conditions. We hypothesize that this is a necessary adaptation for survival in acidic niches and unraveling of this metabolic switch has potential to identify novel therapeutic targets. Studies using 13C tracers showed that leucine is a major source of carbons in these lipids, and we will continue to pursue these lipidomic studies with quantitative flux analyses, and whether inhibiting these anabolic and catabolic pathways shows differential sensitivity to low pH. We will determine if adiposomes are associated with aggressive breast tumors using immune competent HER-2/neu mouse model and human breast cancer tissue microarray. We expect that these experiments will better characterize survival mechanisms under acidosis and identify novel therapeutic vulnerabilities. AIM 1. Determine the mechanisms of signal transduction through acid receptors. Accumulation of lipids as adiposomes is robustly and reversibly induced by exposure of cells to acidic pH. CRISPR/Cas9 mediated depletion of OGR1, but not TDAG8 inhibited acid-induced adiposomogenesis. In Aim 1.1, we will assess if OGR1 depletion affects cell viability, and other responses to acid-adaptation, including autophagy and lysosomal redistribution in vitro, and tumor growth and metastasis in vivo. In Aim 1.2, we will explore signaling downstream of OGR1 and identify transcription factors that regulate adiposomogenesis. AIM 2. Assess the contribution of de novo lipid synthesis and lipid oxidation to acid-induced lipid phenotype. It appears paradoxical that acidosis would stimulate both FAS and βox. 13C tracer experiments show that a major source of carbon for the acyl chains in adiposomes derives from ketogenic amino acids (leucine). In Aim 2.1, we will continue these studies to quantitatively determine the origin of carbon sources for lipids by quantitative flux analyses. In Aim 2.2, we will investigate simultaneous FAS and βox with more granularity using a portfolio of drugs, and inducible shRNA to knock down of anabolic and catabolic enzymes to test if inhibiting these pathways shows differential sensitivity to cells at low pH. AIM 3. Determine if acid-induced adiposomes are associated with aggressive/metastatic breast tumors. In Aim 3.1, this will be interrogated in a HER-2/neu driven murine mammary cancer model by histology. We will neutralize tumor acidity using ad lib bicarbonate and stain tumor sections for Perilipin-2 (adiposomes) and Lamp-2 (acidosis) and analyze the co-occurrence of acidosis and adiposomes on a cell-by-cell basis. In Aim 3.2, we will perform similar analyses on a human breast cancer progression TMA to determine if there is an association of adiposomes with acidosis and disease stage, including metastasis. We expect that the proposed experiments will better characterize this important survival mechanism to unravel novel therapeutic vulnerabilities.

During this reporting period we made progress in our efforts to unravel OGR1 signaling under acidosis. We observed that (endoplasmic reticulum) ER stress induction in response to acid treatment is mediated through OGR1 activation. In addition, we observed that MCF7-NSA cells that are adapted to grow in low nutrient conditions exhibited robust accumulation of lipid droplets compared to control MCF7 cells suggesting that lipid phenotype is an adaptation to nutrient stress.

Follow on Funding: None at the time of reporting.

Collaborations: Dr. Patsy McDonald in the Department of Cancer Physiology at Moffitt Cancer Center to identify small molecule inhibitors of OGR1, a G protein-coupled receptor (GPCR) involved in acid signaling and lipid phenotype.

Dr. McDonald is an expert in GPCR function, regulation and pharmacology and has developed selective small molecule probes for multiple GPCRs using HTS compatible cell based functional assays.

Dr. Timothy Garrett, SECIM, University of Florida and Dr. Matthew Merritt, the Department of Biochemistry and Molecular Biology at University of Florida (expert in Nuclear Magnetic Resonance (NMR) analysis of lipids) are ongoing.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

4. **Grant #:** 8BC06-A1 Therapeutic Targeting of RAGE in Breast Cancer Progression and Metastasis

Principal Investigator: Xiangxi Mike Xu, PhD

Organization: University of Miami

Abstract: The major highlights accomplished in this project include receiving two foundation grant awards to further study the role of RAGE inhibitors in breast cancer initiation (BCRF) and established metastatic disease (METAvivor), presented the research team's work at the AACR San Antonio Breast Cancer Symposium in December 2019, published a patent on the use of RAGE inhibitors in breast cancer, identified novel biological pathways regulated by RAGE in breast cancer cells, established the major biological effect of receptor for advanced glycation end products (RAGE) inhibition is through tumor cell invasion and not proliferation/viability, established the major in vivo dose range of RAGE inhibitors for use in breast cancer models in mice. Essentially, the team has completed and finalized studies as outlined in the proposal. Future plans for this work include developing any new scientific directions and/or taking advantage of new research opportunities/follow-on funding.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at time of reporting.

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

Principal Investigator: Ashok K Saluja, PhD

Organization: University of Miller Miami

Abstract: Metastases or the consequence of their treatment are the biggest contributor to death from cancer. Colorectal cancer is no different. In 2017 about 140,000 people will be diagnosed with colon and rectal cancer in United States. Out of which about 50% will be diagnosed with metastases, either at presentation or during the course of their disease. Thus, there is an urgent need to better understand the process of metastases and develop novel treatment strategies. Unfortunately, our understanding of the process of metastases is still rudimentary. For instance, it is unclear why certain organs are more prone to metastases as compared to others. While mechanical factors such as blood flow and lymphatic drainage pattern are certainly at play, cancer cells demonstrate tropism to certain organs for metastatic growth. In this regard liver is the most common site of liver metastases from colon and rectal cancer. Better understanding of why liver is such a favorable organ for metastases will lead to development of targeted therapies. Liver is believed to be an immune-privileged organ which favors the induction of tolerance than induction of immunity. Whether, this immune-tolerant phenotype contributes to the preponderance of metastases in liver is unknown. Furthermore, the reason why liver is an immunotolerant organ is unclear. Our preliminary data suggest that gut microbiome is responsible for creating an immunosuppressive environment in the liver. In our studies depletion of gut-microbiome with antibiotics prevents growth of liver metastases. We have also observed that depletion of microbiome is unable to inhibit liver tumor growth in a mouse lacking adaptive immunity suggesting that adaptive immune system is required for modulation of liver metastases by microbiome. Furthermore, T-cells obtained from the animals after gut microbiome depletion are very effective in killing cancer cells. Based on this and other literature we have hypothesized that exposure to gut microbial antigens causes immunotolerances and creates a permissive environment for the metastatic colon cancer cells to grow. In the current grant-proposal, we will test this novel hypothesis. In aim 1 using models of colon cancer liver metastases and using antibiotics induced microbiome depletion, use of germ-free mice and use of probiotics we will establish that microbiome modulates liver metastases. In aim 2, using immune profiling and animal experiments we will establish that the reduced liver metastases growth observed on depletion of microbiome is dependent on T cells. And finally, in aim 3, we will evaluate the mechanism by which gut microbiome modulates immune cells in the liver to create an immunosuppressive environment. These innovative studies will provide potential therapeutic breakthrough in treating colon cancer liver metastases by modulating entero-hepatic axis by routine antibiotics, probiotics or by targeting novel pathways identified in this research.

To test if vancomycin could be used as an anti-cancer therapeutic in humans, we designed a series of “avatar- mice” experiments. Here, we recruited patients having colon cancers and collected their stools. Mice were gavaged with broad-spectrum oral antibiotics to totally deplete the murine gut microbiota and then transplanted with stools of deidentified patients making these mice avatar mice with human microbiome. These “humanized” mice were then given either oral vancomycin or no antibiotic and cancer cells were injected subcutaneously. In three out of the five humanized mice, vancomycin significantly reduced the tumor burden suggesting that the gut microbiome modulation could act as an important tumor-modulating strategy in corresponding donor patients.

Further, we have used the stools to analyze the patient microbiome using 16s rRNA sequencing and we plan to analyze the data to further narrow down the identity of cancer promoting vancomycin-targetable bacteria.

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 #:** 8BC09-A1 Multiplex Imaging Resource for State of Florida

Principal Investigator: Eric D Wieder, PhD

Organization: University of Miami

Abstract: The purpose of this grant is to create a highly specialized Imaging Center to allow for highly multiplexed data to be obtained from thin slices of tumor samples or normal tissue. The objective is to develop new treatments for cancer, and to better understand which cancer patients will benefit from a specific treatment, more sophisticated tools are continually being invented. These new technologies allow doctors and scientists to gain increasingly complex information on each person's cancer and will ultimately allow for customize therapy to best benefit each patient and to provide the best possible outcomes. One such tool is the ability to look at tumor samples under the microscope and determine which cell types are in a tumor, what unique markers are on those cells and how other cell types within the tumor are interacting with it. This is done by taking a slice of tumor (biopsy), staining it on a slide and then taking a magnified picture of it using a microscope. There are various staining methods which allow pathology labs to identify various characteristics of tumors, but a more sophisticated way uses antibodies tagged with colors to be able to distinguish different markers on cells within the tumor. In most labs, it is typical to be able to look at one to four markers at the same time, although there is specialized equipment that can look at 10-12 at a time. Recently a technology was developed that uses metal atoms instead of colors to tag and identify each marker, which has increased the number of markers that can be studied simultaneously to 50 markers or more. This technology was commercialized to look at single cells, but not tumor biopsies, within the last decade. More recently, this tool was modified to allow it to work to image cells in a tumor biopsy. Although there are over 60 installations of the recently developed single cell technology at academic and government research centers across the USA, and 30 installations of the new tumor imaging technology across the world, until this proposal was funded, there were none for either single cells or tumors in all of Florida. This disruptive technology has begun to be used by scientists all over the world and results are beginning to be published. Although it took longer than expected to put all the infrastructure in place to provide a world-class imaging facility for users, including an unexpected pandemic, the facility is poised to grow as all the pieces are in place from developing reagents in house, assisting with staining of tissues, image acquisition, and data analysis. The research team is eager to continue to promote the facility

across Florida and are working with counterparts at University of Florida and Moffitt Cancer Center to see if the team can continue to grow the user base across the state. The team will continue to promote utilization of the facility and are pursuing doing an additional round of intramural funding to grow our user base. The team will also continue to stay abreast of technical advances in the field to ensure maximum success of our facility.

Follow on Funding: National Institute of Neurological Disorders and Stroke, Epigenetic pathways and cell cycle exit, Nagi Ayad, $235,000.

NIH NEI, Immune Mechanisms in Ocular Graft vs Host Disease, Victor L. Perez and Dr. Robert Levy $336,000.

Collaborations: A collaboration between investigators from University of Miami and Moffitt Cancer Center has been proposed and a grant submitted.

Journals: None at the time of reporting.

Patents: None at time of reporting.

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

Principal Investigator: Shanta Dhar, PhD

Organization: University of Miami

Abstract: During the second year of the grant, focus has been on synthesizing and troubleshooting the Pamidronate prodrug for encapsulation in the nanoparticle (NP). During this synthesis the research team faced certain challenges as listed below: The PLGA-Pamidronate prodrug molecule as proposed in the grant, was successfully synthesized and encapsulated in the NP. The Plamindronate encapasulated (PLGA-Pamidronate) encapsulated NPs were able to show reduction in invasion/migration of cells. These NPs were able to inhibit osteoclastogenesis. However, the amount of Pamidronate loaded in the NP could not be quantified effectively as the PLGA polymer interfered with the available techniques available to quantify bisphosphonate molecules. Pamidronate is an extremely hydrophilic molecule which requires the team to make a hydrophobic analog in order to encapsulate in to the hydrophobic core of the NP. The hydrophilicity of Pamidronate made standard coupling methods difficult to accomplish. The team was able to successfully synthesize a hydrophobic analog which was able to be quantified easily through High Performance Liquid Chromatography (HPLC), however the yield of the reaction was very low. The team has not faced any major challenges related to shared resources and institutional commitments. The scientific aims of the proposed research remain the same and progress is being made in achieving the goals mentioned in the grant application. During year three of the grant, the team has focused on synthesizing and troubleshooting the Pamidronate prodrug for encapsulation in the nanoparticle (NP). During this synthesis the team faced certain challenges as listed below: The highly hydrophilic nature of pamidronate presents several challenges in synthesis of a new hydrophobic prodrug. The team

was able to develop a luciferase expressing prostate cancer cell line for future in vivo studies. The team obtained tumor tissue for analysis of prostate specific membrane antigen expression. In addition to these, the global COVID-19 pandemic presented several challenges as students and post-docs working on the grant were forced to work from home, preventing meaningful troubleshooting of the reaction conditions to solve the problems presented by the hydrophilic nature of pamidronate. Additionally, due to the pandemic, several institutional core facilities were not operational for a period.

Follow on Funding: None at time of reporting.

Collaborations: None at time of reporting.

Journals: Metabolic modulation of the tumor microenvironment leads to multiple checkpoint inhibition and immune cell infiltration. Kolb, D.; Kolishetti, N.; Surnar, B.; Sarkar, S.; Guin, S.; Shah, S. A.; and Dhar, S. ACS Nano (2020), 14, 11055-11066.

Patents: None at the time of this reporting.

Bankhead-Coley Cancer Biomedical Research Program
Appendix I
Fiscal Year 2020-2021 Completed Grants
Funding Fiscal Year 2016-2017

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
7BC02	University of Florida	Andrew R. Judge, PhD	$1,226,836	12/31/2020	No	Yes	No
7BC05	H. Lee Moffitt Cancer Center	Keiran S.M. Smalley, PhD	$1,468,200	2/29/2020	No	Yes	No
7BC06	Florida Atlantic University	Amy E. Wright PhD	$622,683	2/29/2020	No	Yes	Yes

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

Principal Investigator: Andrew R. Judge, PhD

Organization: University of Florida

Abstract: We found that various cachexia inducing tumor cells, including primary human pancreatic cancer cells, release IL-8 (CXCL8) and/or chemokind C-X-C motif (CXCL)1. Human pancreatic tumor associated stromal (TAS) cells also release IL-8 and CXCL1, and given that cancer cells and TAS cells collaborate within the tumor microenvironment, we further measured both IL-8 and CXCL1 release from cancer cell/TAS cell co-cultures. This identified a synergistic increase in IL-8 but not CXCL1, and this was due to cancer cells stimulating greater release from TAS cells. We have also identified that serum levels of both IL-8 and CXCL1 are increased in cachectic pancreatic cancer patients compared to non-cancer control patients. In agreement with this, a recently published study demonstrated that IL-8 is significantly increased in cachectic compared to non-cachectic pancreatic cancer patients when all patients were considered as a whole, and when resected patients were separately considered or when locally advanced patients were separately considered. Further, the same study showed that IL-8 positively correlated with body weight loss and negatively correlated with muscle mass measured from CT scans and concluded that IL-8 is a characteristic of pancreatic cancer cachexia.

We have further identified that global deletion of CXCR2 is protective against pancreatic cancer-induced cachexia. Indeed, tibialis anterior, gastrocnemius, soleus, and gonadal fat mass were each spared in tumor bearing Cxcr2fl/fl-Cre+ mice compared to Cxcr2fl/fl-Cre- mice. Since Ly6G+ cells are the most prominent source of CXCR2 in mice we also treated pancreatic tumor bearing mice with an anti-Ly6G antibody, or an isotype control. Here we found that tumor bearing mice treated with the anti-Ly6G antibody were protected from cachexia, compared to the tumor bearing isotype control group. Thus, depletion of Ly6G+ cells, the dominant cell type expressing CXCR2, has a similar effect to Cxcr2 deletion in protecting against cachexia. We next determined whether pharmacological inhibition of CXCR2 signaling could inhibit cachexia by treating pancreatic tumor bearing mice with the CXCR2 antagonist, SB225002 (the same

inhibitor used in our in vitro experiments). In these experiments we found that tumor bearing mice treated with SB225002 had significantly larger muscles and significantly more fat compared to vehicle treated tumor bearing mice. Based on these findings, and the knowledge that skeletal muscle expresses CXCR2, we subsequently knocked down CXCR2, using a short hairpin RNA (shRNA) construct, in the skeletal muscle of mice injected orthotopically with pancreatic cancer cells, and found that this abolished the tumor- induced skeletal muscle wasting. These findings suggest that CXCR2 in skeletal muscle is required for the normal muscle atrophy that occurs in response to pancreatic tumors. Since muscle tissue contains multiple cell types, we next questioned whether CXCR2 expression in skeletal muscle cells is required for cancer cachexia. To do this we crossed Cxcr2fl/fl mice with skeletal muscle specific Cre mice (human skeletal actin (HSA)-Cre mice). However, in these experiments we found comparable cachexia in wild type and skeletal muscle specific Cxcr2 knockout tumor bearing mice. Thus, overall, these findings demonstrate that CXCR2 is required for pancreatic cancer cachexia, but that CXCR2 expression in skeletal muscle cells is not required.

To determine whether secretion of the CXCR2 ligands, IL-8 and CXCL1, from pancreatic cancer cells are individually required for cachexia in response to human pancreatic tumors we deleted each from L3.6pl cells and injected wild type (WT), IL-8-/- or CXCL1-/- cells into the pancreas of mice. We found that each of these cell lines induced the same degree of cachexia, suggesting that tumor-derived IL-8 alone and CXCL1 alone do not cause cachexia. However, we did find differences in the serum and tumor profile of cytokines and chemokines, between WT and knock out (KO) cells, and between IL-8 and CXCL1 KO cells. This latter finding suggested that co-deletion of IL-8 and CXCL1 may have additive effects and could attenuate cachexia. To test this, we deleted both IL-8 and CXCL1 from L3.6pl cells and injected WT or IL-8/CXCL1dKO cells into the pancreas of mice. Here, we found that mice bearing WT tumors, but not IL-8/CXCL1dKO tumors, showed significant wasting of skeletal muscle and fat. We subsequently found that time-to-IACUC mandated endpoint was extended by 50% in mice bearing IL-8/CXCL1dKO tumors compared to those bearing WT tumors.

These combined findings identify a central role for the IL-8/CXCL1/CXCR2 axis in the development of cancer cachexia, and thus identify a targetable axis to prevent the development of cachexia in tumor bearing hosts.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Keiran S.M. Smalley, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: Uveal melanoma is the deadliest form of eye cancer. In 50% of cases it metastasizes, overwhelmingly to the liver. Once in the liver, uveal melanoma is almost completely refractory to treatment. The overarching hypothesis of this grant is that the genes responsible for driving uveal melanoma convey a dependency on a class of epigenetic enzymes called the histone deacetylases (HDACs) which might represent a therapeutic vulnerability. The research team further hypothesized that HDACs are required for uveal melanomas to adapt to a potentially promising therapy for uveal melanoma (MEK inhibitors) and that co-targeting of HDACs and MEK will lead to durable therapeutic responses. In this grant the team used state-of-the-art proteomic and gene expression analysis techniques to determine how uveal melanoma cells were able to overcome the effects of MEK inhibitor therapy. Several new mechanisms of therapeutic escape were identified including growth factors directly secreted from the cancer cells, and increased survival signaling that resulted from the cancer cells re-arranging the structure (cytoskeletal re-arrangement). The team determined that these effects were dependent upon HDACs, and that use of HDAC inhibitors (which are in clinical use) could prevent therapeutic escape. The team demonstrated that the combination of a MEK and HDAC inhibitor was effective at shrinking established uveal melanomas in mice. Of note, the team developed a new mouse model of uveal melanoma growing in the livers of mice and showed that the MEK-HDAC inhibitor drug combination was effective against these most intractable of tumors. In parallel to these efforts the team also performed unbiased genetic (CRISPR) screens to identify other targets that could synergize with MEK inhibitors. The purpose of this experiment was to determine if the team could identify targets that were more effective than the HDACs. These screens identified other potential genetic regulators that were then validated alone and in combination with MEK inhibitors in our uveal melanoma mouse models. Among the drug combinations tested, it was found that decitabine (another FDA-approved drug) synergized effectively in mouse models. In addition to tumor intrinsic mechanisms of drug resistance, there is also evidence that normal host cells can also contribute to drug resistance in cancer cells. To evaluate this, the team used a new technology called single cell RNA- Seq which allows the gene expression profiles of individual cells in tumors to be analyzed. Using this approach, the team identified several new pathways and genetic regulators in both human uveal melanoma specimens and mouse tumors that has formed the basis for a new collaborative grant submitted by this group to the National Institutes of Health. Through the course of this grant we have identified several promising new drug combinations. Working with clinical colleagues at Moffitt Cancer Center and the University of Miami the team has planned a new phase II clinical trial of the MEK inhibitor Binemetinib in combination with the HDAC inhibitor Belinostat that should be opening shortly. This trial will lay the ground work for a new family of therapies that offers hope for Floridians with uveal melanoma.

Follow on Funding: None at the time of reporting.

Collaborations: H. Lee Moffitt Cancer Center, The University of Florida and the University of Miami.

Journals: HDAC inhibition enhances the in vivo efficacy of MEK inhibitor therapy in uveal melanoma Faião-Flores, F., Emmons, M.F., Durante, M.A., Kinose, F., Saha, B., Fang, B., Koomen, J.M., Chellappan, S.P., Maria-Engler, S.S., Rix, U., Licht, J.D., Harbour, J.W.,

Smalley, K.S.M.: Clin Cancer Res.(2019) doi: 10.1158/1078-0432.CCR-18-3382. Epub 2019 Jun 21.PMID: 31227503

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

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

Patents: None at time of reporting.

3. **Grant #:** 7BC06-A1 Discovery of Marine Natural Product Antagonists of Survivin as Novel Cancer Therapeutics

Principal Investigator: Amy E. Wright, PhD

Organization: Florida Atlantic University

Abstract: Natural products or their derivatives represent over 48% of clinically approved cancer chemotherapeutics. The HBOI marine natural products chemical library represents a diverse source of genetically encoded small molecules that have actively co-evolved with cellular targets involved in both cell survival and death. The nodal protein survivin has been identified as an important target for intervention in a number of cancers including breast, colon and lung. Its expression correlates to poor prognosis and has a role in the aggressiveness of these diseases. It plays key roles as an antiapoptotic protein; in mitosis; in drug resistance; in angiogenesis and in DNA repair response. Several approaches to inhibit survivin's multiple functions have been explored but there remain very few molecules that inhibit the activity of survivin. Discovery of additional antagonists will advance this field both in our understanding of basic biology of survivin and in clinical practice. The research team hypothesize that assaying chemically diverse natural products in the HBOI library will provide new lead molecules for therapeutic intervention against colon, lung and breast cancers through reducing the level of survivin. The project found an abundance of compounds that can reduce the levels of survivin in cancer cells. Some of the compounds are active at low nM levels while others are active in the low µM which is like many published compounds. Some of the compounds seem to have expected impacts on cell signaling or cell cycle while others showed effects that warrant further work to define how they reduce the levels of survivin. The graduate student is currently studying whether the most active compounds target survivin for degradation by the proteasome. The grant's investigators plan to apply for an RO1 grant from NIH to further explore the mode of action of the compounds and to conduct in vivo analysis. The team also hope to collaborate with appropriate medicinal chemists to optimize structures and to possibly use affinity probes to define the upstream molecular targets.

Follow on Funding: HBOI Foundation, Discovery of survivin-targeting marine natural products from the Indian River Lagoon, Tandberg/McCarthy, $9,250, $10,000, and $19,250.

Collaborations: None at time of reporting.

Journals: Discovery of Survivin Inhibitors Part 1: Screening the Harbor Branch Pure Compound Library. Esther A. Guzmán, Tara P. Pitts, Kirstie, R. Tandberg, Priscilla L. Winder, Amy E. Wright, Marine Drugs.(2020).

Patents: None at the time of reporting.

Bankhead-Coley Cancer Biomedical Research Program
Appendix J
Fiscal Year 2020-2021 Completed Grants
Funding Fiscal Year 2015-2016

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
6BC04	University of Florida	David D. Tran, MD, PhD	$1,226,836	12/31/2020	Yes	Yes	Yes
6BC06	University of Miami	Michael H. Antoni, PhD	$1,468,200	2/29/2020	No	Yes	Yes

1. **Grant #:** 6BC04-A1 Novel Strategies to Target Disseminated Tumor Cells in Triple Negative Breast Cancer

Principal Investigator: David D. Tran, MD, PhD

Organization: University of Florida

Abstract: The original Aims 1 and 2 of this grant were to conduct a Phase 1 and 2 trial testing the concept of using a p38 inhibitor to reawaken low proliferative disseminated tumor cells (lpDTC) in triple negative breast cancer (TNBC) to resensitize them to conventional chemotherapy. Due to the unexpected discontinuation of p38 inhibitor development, the planned clinical trial could not be initiated. To continue the focus on TNBC, the research team had identified IL-6/R, an upstream node of p38. IL-6/R inhibition appears to be more efficient at reawakening lpDTCs. Importantly there are three FDA approved IL-6/R inhibitors. For this reason, the team redesigned the original trial to replace the p38 inhibitor with the commercially available IL-6/R inhibitor sarilumab. The team submitted an NIH/NCI R01 with this concept and received >$2.7 million funding to conduct the trial. The trial was open to enrollment late 2020. The AI platforms that were developed and the funding from the Bankhead Coley grant will be applied to analyze the samples obtained from this new protocol. With Florida Department of Health approval, the team significantly expanded the original Aim 3 over the last two years to focus on the strategic development of AI platforms designed specifically for the analysis and management of Big Data in biomedicine for the University of Florida (UF) and the State of Florida that will be leveraged for the data obtained in the planned R01-funded clinical trial in TNBC for also for other cancers and for biomedical research in general. In the next year the plan is to complete validation of the Virtual Experimentation Framework (VEF). Once done, VEF will represent a powerful AI method to predict master regulator combinations in any biological process and to aid in the development of personalized therapeutics. In the next two years, there is a plan to apply NETZEN and VEF to mine electronic medical records (EMR) to predict health outcomes in human cancers, starting with breast cancer. In the last year, the team has been working with the Breast Pathology group, the IT/Imaging group at UF, and the OneFlorida data trust to create a unique dataset of breast cancer pathological images and mammograms to integrate with a new AI algorithm that the team is developing to mine these large datasets. The current focus is to develop an imaging predictor tool using the mammogram data trust. The next

step is to incorporate the pathological slide images, genomics information and EMR to develop a workable model to predict long-term outcomes in patients with breast cancer, which can then be adapted for other cancers. The team will apply our AI technologies to analyze the entire dataset of the IL-6 Inhibitor trial in triple-negative breast cancer (TNBC) funded by the National Cancer Institutes to mine the global genomics of individual DTCs and lpDTCs to gain deeper understanding of the mechanism of lpDTC development to identify novel targets that can be combined with the IL-6/R/p38 axis to improve DTC-targeting strategies in the near future. The intellectual properties that were developed during this grant, the team plans to seek licensing of the master regulators of glioblastoma (GBM), the cancer-to-immune-cell conversion immunotherapy and NETZEN algorithm to rapidly advance development of these technologies to benefit cancer patients in the state of Florida and the nation and to promote commercialization.

Follow on Funding: NIH/NCI, Novel Methods of Chemo-sensitizing Low-proliferative Disseminated Tumor Cells in Triple Negative Breast Cancer, David Tran, $2,759,466.

NIH/NCI, Targeting Glioblastoma Stem-Like Cells with Custom-Designed Viral Vectors, Falk, Tran, Zolotukhin, $2,000,000 with $156,335 Moonshot supplement.

The Al & Nancy Burnett Charitable Foundation Grant Mechanism, Supporting Research in Dr. David Tran Laboratory, $83,500.

Sasrepta Grant Mechanism , Artificial intelligence-directed approach in human tissue manufacturing for therapeutic targeting, Tran, Zolotukhin, Sawyer, $1,056,398.

2020 AACR-Novocure Tumor Treating Fields Research Grant, Molecular mechanism of resistance to Tumor Treating Fields in glioblastoma, Tran, $250,000.

Collaborations: None at time of reporting.

Journals: None at the time of reporting.

Patents: Tran, D.D. and Chen, D “Methods and Compositions for Determining Susceptibility to Treatment with Checkpoint Inhibitors.” Provisional to be filed March 2021.

Le, S. and Tran, D.D. “Deep Network Systems Calculation of Optimal Ranking Engine (dnSCORE.” Disclosed June 2020: UF T18228

Tran, D.D. “Master Regulators of Breast Cancer Metastasis.” US Provisional Application filed March 2020, Serial No. 62/985,785

Tran, D.D. and Le, S. “Immunotherapy for Direct Reprogramming of Cancer Cells into Immune Cells/Antigen Presenting Cells/Dendritic Cells.” US Provisional Application filed December 2019/PCT file December 2020: No. 62/952,725

Tran, D.D. and Chen, D. “Methods for Reducing Viability of Cancer Cells by Activation of the STING Pathway with TTFields.” U.S. Provisional Application filed November 2019/PCT filed April 2020: Serial No. 16/673,246.

Tran, D.D., Chen, D, and Le, S. “Inhibiting Prostaglandin E Receptor 3 Resensitizes Resistant Cells to TTFields and Prevents Cells from Developing Resistance to TTFields.” U.S. Provisional Application filed March 2019/PCT filed October 2020: Serial No. 62/849,535.

Zolotukhin, S, Tran, D.D., and Kondratov, O. “AAV capsid variants targeting human glioblastoma stem-like cells.” U.S. Provisional Application filed August 2019/PCT filed August 2020: Serial No. 62/884,716 Exclusive licensing agreement to Lacerta Therapeutics, December 2019.

Tran, D.D., and Le, S. “Core Master Regulators of Glioblastoma Stem Cells.” US Provisional Patent Application filed February 2019: Serial No. 62/802,554. Selected one of the 25 finalists globally for the 2019 Brain Race –The Center for Advancing Innovation, Bethesda MD – Licensing company selection pending.

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

Tran, D.D., and Le, S. “Methods for Cancer Screening and Monitoring by Cancer Master Regulators Markers in Liquid Biopsy.” US Provisional Patent Application filed February 2019: Serial No. 62/802,620.

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

Principal Investigator: Michael H. Antoni, PhD and Bonnie B. Blomberg, PhD

Organization: University of Miami

Abstract: Women undergoing breast cancer (BCa) treatment face a multitude of stressors and often experience increased negative affective states (depressed mood, anxiety, anger) and depressive symptoms and decreased positive affect (happiness, contentment), which not only compromise their quality of life but can also contribute to inflammatory signaling, with potential negative health effects over the short- and long-term. Older women (60yrs+) who confront the challenges of BCa treatment may have less coping resources than younger women, resulting in greater negative affect and depressive symptoms. The research team’s prior work also showed that having a poorer Affective status and greater inflammation predicted poorer influenza vaccine (IV) responses in older persons, though this has not yet been demonstrated in older women undergoing treatment for BCa. This study uses a novel technology to deliver stress management intervention groups to older distressed BCa patients through a broadband connection to show for the first time the efficacy of stress management for improving immunological responses to the influenza vaccine (IV) during active primary treatment for BCa. This addresses a major barrier in clinical care—structured interventions delivered in an institutional setting are not feasible for many patients due to physical, logistical, and acceptability barriers. Because these interventions have mostly demonstrated efficacy using a group format, the team will move the field forward by employing technological advances allowing group-based interventions to be delivered in the home. The intervention occurs

between surgery and the start of adjuvant therapy, a period of marked anxiety, and a “moment of opportunity” when patients are motivated for change. This is the first study to test the effects of stress management on responses to IV, and affective and immune/inflammatory processes in a randomized controlled trial using a remotely delivered group Community-Based Social Marketing (CBSM) intervention for older distressed women undergoing treatment for BCa. Use of a home-based delivery system stands to increase the reach of the intervention to older populations in greatest need but who do not use it due to transportation barriers, lack of perceived access, acceptability issues, limited energy, and limited resources. This remote-delivered group CBSM intervention could improve psychological and physiological adaptation in BCa patients during initial treatment period but may also have implications for long-term health outcomes in future work.

Follow on Funding:

SCCC, Video conferenced Stress Management Effects on Affective and Immune Status in Breast Cancer Patients Undergoing Primary Treatment, Michael Antoni, $100,000.

University of Miami Office of Vice Provost of Research and Faculty Affairs, Video conferenced Stress Management Effects on Affective and Immune Status in Breast Cancer Patients Undergoing Primary Treatment, Michael Antoni, $2,500.

Collaborations: University of Miami, Department of Psychology, had 6 graduate students in the Clinical Health Psychology training program and one in the Behavioral Medicine program received training during the project, though their funding was supported elsewhere. They are:

Devika Juagir, Marcella May, Chloe Taub, Molly Ream, Emily Walsh and Erica Nahin, Devika Jutagir completed her PhD studies at University of Miami and served a 3-yr post-doc at Memorial Sloan Kettering Cancer Center, Marcella May continues her PhD training at the University of Miami, Erica Nahin completed her PhD studies at the University and is serving a post-doctoral fellowship at Syracuse VA Medical Center, Chloe Taub completed her PhD studies at University of Miami and is beginning a Post-doc at Northwestern University/Lurie Comprehensive Cancer Center, Molly Ream continues in the PhD program at the University of Miami and obtained an NIH F31 fellowship to support her studies, Emily Walsh continues in the PhD program at the University of Miami supported on a Maytay Fellowship, -University of Miami, Dept of Psychology, initially had two faculty member performing research under the research project: Professor Michael H. Antoni, Ph.D. (M-PI of this grant) and Charles S. Carver, Ph.D.(co-I). Dr. Antoni brings expertise in empirically evaluating stress management interventions in cancer patients and psychoneuroimmunologic processes. Professor Charles Carver, Ph.D., a personality psychologist passed away during YR 4 and Dr. Antoni assumed the responsibilities of Dr. Carver in the measurement of affect and other patient-reported stress processes. Dr. Antoni has many years of experience publishing on the effects of these variables on quality of life, biobehavioral processes and physical outcomes in cancer patients.

University of Miami, Dept of Microbiology/Immunology had two faculty members participating throughout the project, Bonnie Blomberg, Ph.D., Professor (Co-PI of this grant), and Daniella Frasca, Ph.D. Research Associate Professor.

University of Miami, School of Medicine, Department of Surgery had one faculty member performing research: Dr. Susan Kesmodel, Associate Professor of Surgery and Director of the Breast Surgery group at the SCCC and co-Chair of the SCCC Breast Cancer Site Disease Group.

University of Miami, School of Medicine, Dept of Medicine had two faculty members performing research: Dr. Reshma Mahtani and Dr. Daniel O'Neil, both breast cancer medical oncologists who joined the project in January 2020.

Georgetown University, Dept of Medicine had one faculty member performing research: Initially a Professor of Medicine at the University of Miami, Marc Lippman, MD, is now Professor of Medicine, Georgetown University.

Cornell University, Center on Behavior and Aging has one faculty member performing research: Professor Sara Czaja, PhD, previously the Director of UM Center on Aging at the University of Miami, Dr. Czaja is now a Professor at Cornell University.

Journals: Elucidating mechanisms of quality of life disparities in Hispanic women with breast cancer: an examination of disease stage, coping, and affect.Michael H. Antoni Firdaus Dhabhar Ream, M., Pester, M., Goodman,Z., Bainter, S. & Antoni, M.H. (2021).CancerPsycho-Oncology

Patents: None at the time of reporting.

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

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
5BC07	H. Lee Moffitt Cancer Center	Eric B. Haura, MD	$1,686,887	11/15/2020	No	Yes	Yes

1. **Grant #:** 5BC07-A1 Signaling-Associated Protein Complexes for the Molecular Annotation of Therapeutic Vulnerabilities, Resistance-Associated Signaling & Tumor Heterogeneity in Lung Cancer

   Principal Investigator: Eric B. Haura, MD

   Organization: H. Lee Moffitt Cancer Center

   Abstract: Attacking aberrantly receptor tyrosine kinases (RTK) is the standard of care for certain cancers, such as subtypes of lung cancer driven by genetically altered Epidermal Growth Factor Receptor (EGFR) and echinoderm microtubule-associated protein-like 4-Anaplastic lympohma kinase EML4-ALK. While genomic alterations in RTK identify patients likely to respond, the underlying molecular mechanisms that drive the magnitude of response as well as the duration of response remain unclear. One mechanism of resistance to kinase inhibitors is through bypass mechanisms that retain downstream pathway activation and cell survival. A major challenge in the field is to accurately identify bypass RTK in individual tumors accounting for both the tumor genome and the tumor stroma. Activation of RTK leads to assembly of protein complexes on RTK that facilitate downstream signal transduction. The research team has exploited this known mechanism to develop proximity ligation assays (PLA) to annotate EGFR protein complexes, a key RTK in lung cancer and other cancers. The team showed these assays reflect EGFR kinase activity both in vitro using cancer cell lines and in vivo using mouse xenograft models of lung cancer. The human tumor data shows high PLA signal in tumors with activating EGFR mutation as expected but interestingly the team can identify tumors with high PLA signal lacking EGFR mutation or that have KRAS mutation. The team propose to extend these findings to study how RTK protein complexes are produced in cooperation through the genome and tumor stroma. Assessment will be made of these RTK complexes for their influence on drug sensitivity. Specific Aim 1 will develop PLA assays reflecting activated MET and FGFR complexes and optimize imaging strategies. Specific Aim 2 will characterize the influence of RTK protein complexes on drug sensitivity in cellular and mouse models of lung cancer. Specific Aim 3 will characterize patterns of activated RTK complexes in human lung cancer tissues and their role in kinase inhibitor sensitivity and resistance. The team is currently submitting a manuscript supported by this grant that describes AXL signaling mechanism, AXL Receptor Tyrosine Kinase(AXL) signaling associated protein complexes, chemical biology of AXL targeting agents, and the role of AXL in EGFR TKI

mediated drug resistance. As a continuation of our investigation into AXL signaling biology in the context of TKI resistance, the team will use BioID to investigate differences in AXL-adapter coupling that can mediate bypass resistance. The team has also submitted and addressed peer review comments on the manuscript submitted to Clinical Cancer Research. The team believes that data generated from this grant is providing a strong mechanistic rationale to utilize the application of mass- spectrometry based proteomics and PLA in clinical settings to aid personalized approach to cancer care. The team believes that PLA-based approach has potential to assess RTK protein complexes directly in-patient samples and assessing their role in modulating KRASG12C inhibitors sensitivity and resistance. The future goals are to utilize novel EGFR:GRB2 & MET:GRB2 PLAs, develop new PLAs to assess complexes indicating activation of specific RTKs into KRAS mutants that could affect drug sensitivity. Based on the results, the team would like to establish HER2:HER3 and FGFR1:FRS2 PLA assays to predict clinically effective combination strategies in diverse group of KRASG12C mutant lung cancer. Moreover, in collaboration with Revolution Medicines, we will evaluate the role of EGFR and MET PLA assays to predict efficacy of KRAS specific inhibitors.

Follow on Funding: Revolution Medicines, Inc., Exploring correlation between EGFR/MET activation status and RMC-4630 sensitivity in tumor tissue using PLA assays, Eric Haura, $238,754.

Revolution Medicines, Inc., Exploring correlation between RTK activation status and KRAS G12C(ON) inhibitor sensitivity, and developing new PLA assays to inform RAS and mTOR pathways, Eric Haura, $275,906.

NCI, Validation of EGFR Protein Complexes as Molecular Diagnostics, Eric Haura, $993,537.

Collaborations: Work has formed preclinical collaborations with Janssen Pharmaceuticals and more recent Revolution Medicines. The later collaboration started late in 2020 and will evaluate the role of EGFR and MET PLA assays to predict efficacy of KRAS specific inhibitors. Our work in PLA also fostered collaborations new funded through a NCI U01.

Journals: Cell-type Specific Adaptive Signaling Responses to $KRAS^{G12C}$ inhibition. Hitendra S. Solanki, Eric Welsh, Bin Fang, Victoria Izumi, Lancia Darville, Brandon Stone, Ryan Franzese, Sandip Chavan, Fumi Kinose, Denis Imbody, John Koomen, Uwe Rix, Eric Haura. Clinical Researcher Cancer. (2021).

Proteomic Characterization of AXL Kinase Inhibitors and Signaling Pathways. Anurima Majumder, Sina Hosseinian, Mia Stroud, Emma Adhikari, James J. Saller, Matthew A. Smith, Guolin Zhang, Shruti Agarwal, Benjamin S. Meyer, Fumi Kinose, Kiah Bowers, Bin Fang, Paul Stewart, Eric A. Welsh, Theresa A. Boyle, Aaron S. Meyer, John M. Koomen, and Eric B.Haura. Cancer Research. (2021).

Cell Signaling Heterogeneity is modulated by both cell intrinsic and extrinsic mechanisms: an integrated approach to understanding targeted therapy. Eunjung Kim, Jae-Young Kim, Matthew A. Smith, Eric B. Haura, Alexander RA Anderson. PLOS Biology.

MET-GRB2 Signaling-Associated Complexes Correlate with Oncogenic MET Signaling and Sensitivity to MET Kinase Inhibitors. Matthew A. Smith, Thomas Licata, Aliya Likhani, Marileila Varella Garcia, Hans-Ulrich Schildhaus, Vincent Vuaroqueaux, Balazs Halmos, Alain C. Borczuk, Y. Ann Chen, Benjamin C. Creelan, Theresa Boyle, Eric B. Haura. Clinical Cancer Research.

EGFR mediates responses to small molecule drugs targeting oncogenic fusion kinases. Stephen B. Keysar, Magdalena J. Glogowska, Matthew A. Smith, Severine Kako, Natalia J. Sumi, Kurtis D. Davies, Kathryn E. Ware, Marileia Varella- Garcia, Eric B. Haura, Antonio Jimeno, Lynn E. Heasley, Dara L. Aisner, and Robert C. Doebele. Cancer Research.

Patents: None at the time of reporting.

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

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
21K01	University of Miami	Yanbin Zhang, PhD	$100,000	11/30/2021	No	No	No
21K02	University of Miami	Robert Starke, MD	$535,840	4/30/2026	No	No	No
21K03	University of Florida	Daiqin Liao, PhD	$535,840	4/30/2024	No	No	No
21K04	H. Lee Moffitt Cancer Center	Christine Chung, MD	$1,339,540	4/30/2026	No	No	No
21K05	University of Miami	Carlos Moraes, PhD	$535,840	4/30/2024	No	No	No
21K06	University of Miami	Helen M Bramlett, PhD	$535,840	5/31/2024	No	No	No
21K07	University of Miami	Scott Welford, PhD	$528,130	4/30/2024	No	No	No
21K08	University of Florida	Rajesh Mohandas, MD, MPH	$535,840	4/30/2024	No	No	No
21K09	Florida International University	Hoshang Unwalla, PhD	$535,680	6/30/2024	No	No	No
21K10	University of South Florida	RELINQUISHED					
21K11	University of Florida	Chengguo Xing, PhD	$1,114,480	4/30/2026	No	No	No
21K12	Florida State University	Michelle Parvatiyar, PhD	$535,396	6/30/2024	No	No	No
21K13	University of Miami	Adam Wanner, MD	$600,000	06/30/2024	No	No	No

1. **Grant #:** 21K01 Defining Role of FANCA in Genome Instability

   Principal Investigator: Yanbin Zhang, PhD

   Organization: University of Miami

   Abstract: One of the most predominant hallmarks driving cancer development is genome instability. It creates genome-wide diversity that enables cells to acquire additional capabilities required for cancer development and progression. Most of the ~400 genes known to be mutated and implicated in cancer development are a direct result of increased genome instability. Therefore, understanding the molecular mechanisms of genome instability in cancer cells is imperative for the development of novel treatment strategies. Fanconi Anemia (FA) is a hereditary disorder caused by mutations in at least 22 genes and clinically characterized by bone marrow failure and predisposition to cancer. During the preliminary studies, we found that FANCA promotes error-prone DNA repair that drives genome instability; its expression is upregulated in many cancer types, and the expression level is strongly associated with breast cancer progression and inversely correlates with breast cancer patient survival. Intriguingly, FANCA recruitment to double strand breaks and DNA damage sites requires active transcription in a live cell analysis. More importantly, knockout of FANCA in a triple negative breast cancer cell MDA-MB-231 initiates cell cycle arrest and cellular senescence and abolishes breast cancer

formation in mice. Based on these preliminary data, we hypothesize that high expression of FANCA in cells (e.g. due to tobacco-induction) promotes genome instability and cancer development and FANCA serves as a vulnerability for cancer treatment. To delineate the role of FANCA in genome instability and cancer development, we will use a biochemically defined in vitro system, a transcription-coupled Double Strand Break (DSB) repair reporter system, a live cell imaging system, a xenograft mouse model, and genome-wide instability analysis to accomplish three aims: Aim 1 is to determine the molecular mechanism of how FANCA contributes to R-loop-mediated genome instability; Aim 2 is to study the role of FANCA in DSB-mediated genome instability and how FANCA is regulated; Aim 3 is to determine the relationship between FANCA-mediated genome instability and cell cycle progression. Completion of this proposal will define a novel role for FANCA in genome instability. This work will also elucidate the significance of FANCA as a unique, rationale-driven target for breast cancer treatment. The outcome of this proposal will expand treatment strategies for breast cancer patients with elevated FANCA expression and genome instability.

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 #:** 21K02 Cigarette Smoke Induces Endothelial Dysfunction Leading to Cerebral Aneurysm Pathogenesis

Principal Investigator: Robert Starke, MD

Organization: University of Miami

Abstract: Cerebral aneurysm (CA) is a vascular disorder in which weakening of the intracranial arteries causes localized dilation or bulging of the blood vessel wall. If left untreated CA may rupture resulting in a subarachnoid hemorrhage, a devastating form of stroke which causes significant morbidity and mortality. Therefore a large number of CA patients are treated prior to rupture by either open surgical or endovascular techniques. Despite treatment approximately 40% of aneurysms will recur and approximately 20% of patients will require retreatment. The reason for CA formation and rupture is not fully understood. However, risk factors for CA formation and rupture have been identified and include increasing age, female sex, hypertension, excessive alcohol intake and smoking. Of these risk factors cigarette smoking is the most significant modifiable behavior associated with CA formation, rupture and even aneurysm treatment failure. The mechanisms by which cigarette smoke causes CA formation, rupture, and treatment failure is not fully defined.Cellular dysfunction and death of endothelial and vascular smooth muscle cells, the two primary cellular components of the blood vessel wall, are hallmarks of CA pathophysiology. Using experimental aneurysm models our laboratory and others has demonstrated that cigarette smoke exacerbates vascular smooth muscle dysfunction which not only increases the incidence of CA formation but also CA rupture. Cigarette smoke is

also known to alter normal endothelial function and even induce cell death. However, it is not known if cigarette smoke induces endothelial cell dysfunction which in turn increases the likelihood of aneurysm formation. Therefore the over-all objective of this grant is to determine the effects of cigarette smoke on endothelial function during CA formation, progression, and rupture. This objective will be investigated using a mouse cerebral aneurysm model and endothelial cells biopsied from human aneurysm tissue. In our first aim, we will determine the effects of cigarette smoke on endothelial function during CA formation in an experimental aneurysm model. In our second aim we will determine if cigarette smoke alters the genomic and proteomic profiles of endothelial cells obtained from aneurysm tissue of non-, former, and current smokers undergoing CA repair. Together these aims will address the clinically relevant question of why cigarette smoke is associated with increased CA risk and treatment failure. The ability to obtain human endothelial cell biopsy's combined with a mouse CA model makes this study unique and innovative and will allow for the identification of important molecular processes altered by cigarette smoke that lead to endothelial loss and enhanced aneurysm formation.

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 #:** 21K03 Novel Mechanism of Action and Translational Potential of the HDAC Inhibitor SR-4370 for Treating Breast Cancer

Principal Investigator: Daiqin Liao, PhD

Organization: University of Florida

Abstract: Breast cancer is the leading cause of cancer burden for women, diagnosed in over 1 million worldwide each year. Breast cancer affects one in 20 women globally and as many as one in eight in Western countries. Although more and more patients with breast cancer have survived of the disease, over 450,000 patients die of this disease annually. About one third of invasive breast cancers progress to recurrent or metastatic disease, and ~90% of breast cancer deaths are due to metastatic cancer in vital distant organs such as brain, liver and lungs. There are several major breast cancer subtypes: estrogen receptor-alpha positive (ER+), HER2-enriched and triple-negative (TNBC). All subtypes can progress to distant metastases. Metastatic breast cancer is currently incurable. The short median survival of 3 years for patients with metastatic breast cancer has not significantly changed in over 20 years. Therefore, more effective treatments are urgently needed to combat breast cancer. Histone deacetylases (HDACs) are enzymes that catalyze biochemical reactions important for cancer cell proliferation. Through analyzing data from thousands of breast cancer patients, increased production of HDACs in cancer was found to correlate with treatment resistance and shortened survival. Fortunately, HDACs are "druggable" targets. Thus, drugs that inhibit HDACs can be developed for treating recurrent and drug-resistant breast cancer. Indeed, HDAC inhibitors have been

tested for treating breast cancer patients in the clinic, but so far, no HDAC inhibitor has been approved by FDA for treating this disease. Current HDAC inhibitors are designed to inhibit HDAC's enzymatic activity. The ineffectiveness of these inhibitors suggests that inhibition of HDAC enzymatic activity alone may not be sufficient to kill cancer cells. Drug candidates with novel mechanisms of action to ablate HDAC functions may lead to more effective treatment. In this project, The PI's lab has discovered novel HDAC inhibitors that not only inhibit HDAC's enzymatic activity, but also degrade HDACs. Drugs with such dual mechanisms of action have not discovered before. The specific goal of this project is to test the novel HDAC inhibitors for their effectiveness in suppressing the growth of breast tumors as well as their metastasis to other organs using breast cancer animal models that closely mimic breast cancer in humans. The new HDAC inhibitors are small-molecule compounds and are thus suitable for various systemic treatments, such as via oral administration. Because the new agents are highly selective to inhibit HDACs, they are less likely to hit other targets in the human body, and thus are expected to be safer than less selective drugs. It is anticipated the novel HDAC inhibitors to be tested in this project may lead to an effective therapy to increase the survival of women with advanced breast cancer.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

4. **Grant #:** 21K04 Effects of Hypoxia in Tumor Immune Microenvironment in Tobacco-Related Head and Neck Squamous Cell Carcinoma (HNSCC)

Principal Investigator: Christine Chung, MD

Organization: H. Lee Moffitt Cancer Center

Abstract: Head and neck squamous cell carcinoma (HNSCC) remain one of the most devastating cancers in the United States. Common risk factors are tobacco and alcohol use and human papillomavirus (HPV) infection. HPV-positive patients have a favorable outcome given current standard of care compared with patients with HPV-negative, tobacco-related HNSCC. Even within HPV-positive HNSCC, patients with a history of tobacco use have worse outcome compared to HPV-positive patients without smoking history, and their disease course resembles tobacco-related HNSCC. Recently immunotherapy has become a promising therapeutic option in HNSCC. Programmed cell death-1 (PD-1) is one of the clinically significant checkpoint molecules that suppress T-cell function upon binding to its ligands, PD-L1 and PD-L2, which are expressed on tumor cells. However, patients with recurrent and/or metastatic (R/M) HNSCC have median overall survival of 7.5-13 months given immunotherapy with/without chemotherapy. Therefore, development of new therapeutic options for these patients is urgent. Increasing evidence suggests that the tumor microenvironment of tobacco-related HNSCC is immunosuppressive, in part due to hypoxia. The tumor microenvironment includes both anti-

tumor effector cells (CD8+ cytotoxic T lymphocytes, CD4+ helper T cells, natural killer [NK] cells) and regulatory/suppressive immunocytes such as regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC). Other factors, such as local concentrations of cytokines and chemokines including VEGF also play a role in determining the environment. The balance of effector and regulatory cells determines whether an individual tumor microenvironment is associated with productive anti-tumor immunity or localized immunosuppression and unrestrained tumor growth. Recent data support the immunosuppressive roles of VEGF including inhibition of dendritic cell maturation, accumulation of MDSC, development of tumor-induced macrophages, induction and maintenance of Tregs, and modulation of inhibitory checkpoint proteins leading to T-cell exhaustion. In addition, a recent study revealed that the presence of myeloid lineage immune cells in inflamed tumor resulted in poor prognosis compared to the presence of lymphoid lineage immune cells in HNSCC. While preclinical and clinical evidence of anti-angiogenesis as an effective therapeutic option for HNSCC patients is existing, we will evaluate an anti-angiogenic agent, cabozantinib, in context of immunotherapy in hypoxic HNSCC. In this proposal, we propose to: 1) determine the effects of angiogenesis inhibition by cabozantinib in reversal of hypoxia and subsequent restoration of proinflammatory tumor microenvironment using in vivo syngenetic mouse model (assessment of immunogenic cell death and ELISPOT) and characterization of additional druggable genes/pathways. using multi-omics, 2) determine the synergy between cabozantinib and anti-PD1 mAb and characterize cytokines/chemokines in the tumor microenvironment of hypoxic tumors using in vivo syngenetic mouse model, and 3) identify biomarkers of response given a combination of cabozantinib and pembrolizumab in tumors collected from an ongoing phase II clinical trial in patients with R/M HNSCC. We will classify the tumors based on the degree of hypoxia and immune cell infiltration and interrogate the molecular differences using multiplex immunohistochemistry and multi-omics.

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 #:** 21K05 Mechanisms of Mitochondrial DNA Deletion Formation

Principal Investigator: Carlos Moraes, PhD

Organization: University of Miami

Abstract: One of the most deleterious effect of cigarette smoking is the increase of oxidative damage to cells in the body, particularly (but not exclusively) in the lungs. Oxidative stress damages different cellular organelles, including the mitochondria, which leads to further damage to the cell. The mitochondrial DNA (mtDNA) is also a target of oxidative damage, manifested mostly as single or double-strand breaks of the DNA. We have shown that when the normally circular mtDNA is fragmented, the linear pieces are rapidly degraded, by a mechanism that

involves the exonuclease activity of the mitochondrial DNA polymerase gamma (PolG). PolG is responsible for replicating the mtDNA, but its exonuclease activity can correct base misincorporations during replication. This conclusion was reached by inactivating the exonuclease activity of PolG in mice (PolG exo-), which caused fragmented mtDNA to not be degraded at normal rates. Linear fragments lingered in the mitochondria for prolonged periods of time, leading to re-circularization and formation of mtDNA deletions. We showed that this occurs (in vitro and in vivo) when the mtDNA is cleaved by a mitochondrial-targeted restriction nuclease (a transgene coding for mitoPstI) in a PolG exo- background. MtDNA deletions are known to cause diseases and to accumulate in postmitotic tissues of normal individuals as they age. Therefore, the understanding of how mtDNA deletions occur and how their formation is exacerbated by oxidative stress and cigarette smoking is of major important to prevent and treat tobacco-related diseases. In this proposal, we will test whether oxidative stress (generated in a mouse model by knocking out one copy of the superoxide dismutase 2 gene) increases the formation of mtDNA deletions in combination with PolG exo-/mitoPstI, providing direct evidence in an in vivo model that oxidative stress enhances mtDNA deletion formation. We will also directly test whether cigarette smoke in mice with a PolG exo-/mitoPstI leads to the increased formation of mtDNA deletions. Finally, we will take advantage of these models to create a mouse with high levels of mtDNA deletions by expressing a transgene for a mitochondrial-targeted DNA ligase. Such model would allow us to test the phenotypic consequences of high levels of mtDNA deletions

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 #:** 21K06 Post-Stroke Combination of Therapeutic Hypothermia (TH) and Whole Body Vibration (WBV) Improves Cognition in Nicotine- Exposed Rats

Principal Investigator: Helen M Bramlett, PhD

Organization: University of Miami

Abstract: Cigarette smoking is a preventable risk factor for stroke which is one of the leading causes of death and disability in the USA. There are three main types of stroke: transient ischemic attack, hemorrhagic stroke and acute ischemic stroke and the latter constitute 87% of total stroke cases. Currently, the only clinical therapies available for acute ischemic stroke are thrombolysis (tPA) and the newly developed method of mechanical endovascular recanalization, both of which have only limited applications in a small number of patients. Among stroke survivors, cognitive decline is one of the most significant problems where almost two-thirds experience cognitive deficits that last at least up to 6 years post-stroke. Therefore, identifying successful novel neuroprotective strategies to improve post-stroke cognition remains a high priority. In animal models of stroke (transient middle cerebral artery occlusion – tMCAO),

behavioral studies in addition to data on infarct volume, motor, and sensory dysfunction, provide evidence of deficits in spatial, non-spatial, and motor learning. These deficits can be minimized with whole body vibration (WBV), a noninvasive therapy in middle-aged rats. On the other hand, long-standing research from our laboratory has shown that therapeutic hypothermia (TH) is a potent neuroprotective therapy in experimental cerebral ischemia with multiple effects at several stages of the ischemic cascade. Therefore, the goal of the current proposal will be to test the efficacy of combined TH and WBV on post-stroke cognitive improvement in nicotineexposed rats of both sexes. If successful, our study will identify a novel therapeutic approach for stroke rehabilitation.

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 #:** 21K07 Chemerin: A Link Between Obesity, Smoking, and Renal Cancer

Principal Investigator: Scott Welford, PhD

Organization: University of Miami

Abstract: Advanced clear cell renal cell carcinoma (ccRCC) leads to death within five years for nearly 90% of patients due to poor responses to current therapies; thus new therapeutics are sorely needed. Cigarette smoking, obesity, and hypertension are the most well established risk factors for ccRCC, and the histology of clear cell tumors give intriguing clues to its etiology. ccRCCs display obvious signs of fat transdifferentiation, such as gross lipid and glycogen-rich cytoplasmic deposits. We recently identified a mechanism driving lipid deposition, and showed that preventing lipid storage restricts tumorigenesis. The data suggests that targeting metabolism holds therapeutic promise. Fat cells, or adipocytes, secrete a variety of signaling molecules (adipokines) that are essential for maintenance of adipose tissue and lipid storage, but that can also contribute to several tumor phenotypes. Some of the proposed mechanisms connecting excess body fat and cancer include chronic inflammation, oxidative stress, and dysregulated oncogenic signaling, all of which can be theoretically linked to disrupted adipokine homeostasis. With the observed transdifferentiation phenotype of ccRCC, and the association with obesity, we reasoned that both autocrine (from tumor) and/or paracrine (from adipose) adipokine signaling could play roles in regulating lipid metabolism and tumorigenesis in ccRCC. Importantly, adipokines have potential as pharmacological targets. We thus undertook an in silico approach to identify potential adipokines that may have a role in ccRCC, and identified the adipokine Chemerin as highly attractive. Chemerin is overexpressed in ccRCC and in obesity, controls lipid metabolism, and is prognostic for outcome. Strikingly, Chemerin is also epigenetically regulated by smoking. Thus it is hypothesized that Chemerin is an obesity and smoking related driver of renal cancer development, and propose that inhibition of Chemerin could have significant therapeutic benefit.

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 #:** 21K08 Endothelial Circadian Clock Protein PER1 Modulates Salt-sensitive Hypertension

Principal Investigator: Rajesh Mohandas, MD, MPH

Organization: University of Florida

Abstract: Smoking leads to high blood pressures and cardiovascular disease. The molecular clock, which controls most bodily rhythms, is affected by smoking and is an important regulator of blood pressure. We have identified that in the kidney, the clock protein Period Circadian Regulator 1 (PER1) modulates Endothelin-1 (ET-1) levels to affect salt reabsorption. However, although genetic deletion of PER1 increases blood pressures, it has no effect on salt handling by the kidney, ET-1 is a potent vasoconstrictor acting through its receptors ETA and ETB. The effect of PER1 on the vasculature has not been evaluated so far.We hypothesize that PER1 in endothelial cells modulates systemic vascular resistance and salt-sensitive hypertension. It was recently identified that a novel long non-coding RNA, EDN-1 AS, that is regulated by PER1 and increases ET-1 mRNA and protein levels. The hypothesis is that PER1 modulates EDN- 1 AS to influence ET-1, vasoreactivity, systemic vascular resistance and blood pressures. There are three specific aims. Aim 1: To determine the extent to which endothelial cell PER1/ET1/ETA/B axis mediates systemic vascular resistance and salt sensitive hypertension Endothelial cell specific PER1 Knock Out (KO), PER1/ET-1 KO, PER1/ETA KO, PER1/ETB KO mice will be subject to normal diet or HS/DOCP treatment. BP will be measured by telemetry. Systemic vascular resistance will be measured in vivo using echocardiography. Aim 2: To determine the role of endothelial cell PER1/ET1/ETA/B axis in mediating vasoreactivity and myogenic tone. Mice with vascular endothelial cell specific deletion of PER1, PER1/ET-1, PER1/ETA and PER1/ETB will be subject to normal diet or HS/DOCP. Vasoreactivity and myogenic tone will be assessed by pressure myography of mesenteric vessels. Aim 3: To determine the molecular mechanism by which PER1 modulates EDN-A1S and ET-1 in enodthelial cells. Our hypothesis predicts that EDN1-AS acts as a chromatin modifier /microRNA sponge to increase ET-1 mRNA. We will examine this using our novel cellular model of EDN1-AS overexpression, PER1si RNA, EDN1-AS /chromatin interactions and microRNA mediated degradation of ET-1mRNA. PER1 could be a novel therapeutic target for hypertension. The clinical development of ET-1 antagonists has been limited by side effects. Targeting PER1 or EDN1-AS might be a novel way to antagonize ET-1 in a tissue specific manner for therapeutic purposes.

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 #:** 21K09 Pathophysiological Mechanisms and Therapeutics for Chronic Lung Inflammation in Smokers and in Chronic Obstructive Pulmonary Disease (COPD)

Principal Investigator: Hoshang Unwalla, PhD

Organization: Florida International University

Abstract: The long-term goal of this proposal is to mitigate chronic lung inflammation and aging in smokers and Chronic Obstructive Pulmonary Disease (COPD). Tobacco smoke and inhalation of particulate matter are the main causes of lung inflammation and aging in COPD. The Cystic fibrosis transmembrane conductance regulator (CFTR) and airway lactoperoxidase (LPO) constitutes the CFTR-LPO axis which plays a pivotal role in scavenging airway hydrogen peroxide (H2O2), consequently producing the hypothiocyanite ion which mediates antibacterial effects in the airway. Our preliminary data show that chronic H2O2 elevation suppresses mitophagy and promotes epithelial cellular senescence. Epithelial cell senescence, and the consequent senescence-associated secretory phenotype (SASP), plays a central role in lung inflammation and the pathogenesis of COPD. Hence suppression of any component of the CFTR-LPO axis can elevate airway H2O2 with multi-factorial effects on mucociliary clearance, the lactoperoxidase-thiocyanate pathway (leading to recurrent lung infections), impaired mitochondrial function, and senescence. Transforming growth factor -beta (TGF-β) signalingis increased in COPD patients and cigarette smokers. TGF-β signaling and cigarette smoke (via TGF-β signaling) alters alter the bronchial epithelial microRNAome and elevates miR-145-5p and miR-449b levels. This leads to suppression of both CFTR and LPO. Aim 1 will identify the mechanism by which HIV Tat, Cigarette smoke and TGF-β signaling suppresses airway lactoperoxidase and determine the impact of the consequent H2O2 upregulation on lung inflammation in vitro and in vivo. Aim 2 will test a novel gene specific microRNA antagonism approach to preserve CFTR function in combination with exogenously delivered recombinant LPO to restore baseline H2O2 and prevent inflammation in the context of cigarette smoke.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

10. **Grant #:** 21K11 Reducing Tobacco-Associated Lung Cancer Risk: A randomized Clinical Trial of AB-Free Kava

Principal Investigator: Chengguo Xing, PhD

Organization: University of Florida

Abstract: Tobacco use is the leading cause of numerous preventable diseases in the United States. Nearly half a million American adults die prematurely of tobacco exposure each year. About 80 – 90% of lung cancer incidence is caused by tobacco use and lung cancer deaths contribute to 29% of tobacco-associated deaths nationwide. In Florida, nearly $10 billion is spent on healthcare cost due to smoking each year while there are still around 32,000 adults die from smoking-related illness each year with nearly 12,000 of them due to lung cancer. Tobacco cessation, therefore, is of critical importance to mitigate this devastating health issue. Quitting, however, is challenging due to the addictive nature of nicotine in tobacco products. Many smokers do not succeed even after multiple attempts with standard pharmacological and psychosocial interventions. Therefore, there is an urgent need of novel interventions to facilitate tobacco cessation. If such an intervention can simultaneously reduce the risk of tobacco-associated diseases, such as lung cancer, that will be even more desirable. Kava is a dietary supplement on the US market that reduces stress and promotes relaxation. It has demonstrated to reduce anxiety and improve sleep. These properties all support kava's potential to facilitate tobacco cessation, but they have not been previously evaluated. Epidemiological and our data also suggest kava's potential to reduce cancer risk, particularly for tobacco-related lung cancer. Built upon these data, we completed a pilot pre-post one-week kava intervention trial and demonstrated that even short-term kava use reduced tobacco use and tobacco dependence and reduced lung carcinogenesis risk. The trial had a high kava compliance rate with no signs of adverse effects. We therefore hypothesize that kava is a novel, safe, and effective candidate in facilitating tobacco cessation and reducing tobacco-associated diseases, including lung cancer risk. To test this hypothesis, we will perform a double-blinded, randomized, placebo-controlled four-week kava clinical trial to a) validate the effect of kava on reducing tobacco use/dependence and explore its sustainable effects; b) demonstrate the kava effect in reducing lung cancer risk; c) interrogate the mechanisms of action; d) in an exploratory fashion, examine potential subgroups most likely benefit from kava intervention; and e) rigorously monitor safety and compliance of kava use. The findings, if supportive of our hypothesis, will be foundational for the design of a randomized efficacy clinical trial to rigorously optimize kava's benefits, including treatment regimen and identifying the targeted populations, which will pave the path to its eventual application among the high-risk populations to effectively reduce tobacco-associated illness.

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 #:** 21K12 Determining How Tobacco Use and Obesity Exacerbates a Novel Cardiovascular Risk Factor

Principal Investigator: Michelle Parvatiyar, PhD

Organization: Florida State University

Abstract: The increased worldwide incidence of obesity is frequently accompanied by poor lifestyle choices such as smoking increases the risk of cardiovascular disease and cancers. The aim of this proposal is to investigate the role of sarcospan (SSPN) in governing inflammatory and metabolic pathways that contribute to cardiometabolic disease and adverse cardiovascular outcomes. Human genome-wide association studies (GWAS) have previously identified the SSPN gene as an obesity susceptibility locus, specific mutations influence SSPN expression and susceptibility to cardiometabolic disease. Preliminary data shows that exposure of wild-type (WT) mice to cigarette smoke (CS) alters SSPN expression, which has been shown to have an emerging role in gating metabolic and inflammatory activation. Total body ablation of SSPN in mice (SSPN KO) causes distinct cardiovascular susceptibility to ischemia-reperfusion (I/R) injury, myocardial infarction, and ventricular arrhythmias. Interesting, further investigation of SSPN in metabolism indicates that SSPN ablation protects against diet-induced obesity and development of insulin resistance. Examination of SSPN KO tissues after high fat diet revealed a marked reduction in ectopic fat deposition and inflammation compared to WT tissues. In essence, we have shown that SSPN ablation confers a lean phenotype and protects against chronic inflammation induced by obesity. The team posits that SSPN has an unknown modulatory role in immunometabolism that maintains metabolic homeostasis and limits natural cardiac responses to injury. It is hypothesized that smoking undermines the normal regulation of SSPN, especially under chronic nutrient excess and that low SSPN expressing individuals may be at even greater cardiovascular risk. The absence of SSPN appears protective against obesity-induced “low grade inflammation” in white adipose and other tissues. However, SSPN KO bone marrow-derived macrophages exhibit greater Type I IFNγ responsiveness leading us to suggest that SSPN is a negative regulator of the pathway. Preliminary data supports our central hypothesis that SSPN deficiency safeguards from cardiometabolic disease under obesiogenic conditions by disrupting normal signals that drive obesity-associated inflammation, but it also potentiates the severity of myocardial infarction by enhancing activation of innate pathways that respond to tissue injury. It is anticipated that smoking further potentiates this activation. SSPN-null and WT mice will be subjected to diet-induced obesity by high-fat diet (HFD) and/or cigarette smoke (CS) to examine how SSPN ablation impacts inflammation under “pro-inflammatory” conditions and increases the severity of cardiovascular disease. The team will examine the impact of smoking on atherosclerotic development in SSPN KO mice and cardiac remodeling and function. Immune cell populations in these mice after CS and/or HFD will be examined by proliferation and flow cytometry studies. To translate our findings to humans we will examine inflammatory profiles and SSPN expression in peripheral blood mononuclear cells obtained from individuals with distinct clinical risk factors. In this application our hypothesis will be tested at various levels including the whole animal, tissue, cellular and molecular levels to examine how smoking impacts SSPN regulation of immune function under normal diet conditions and also under the influence of nutrient excess.

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 #:** 21K13 Early Detection of Vaping-Related Vascular Disease

Principal Investigator: Adam Wanner, MD

Organization: University of Miami

Abstract: It has been shown that cigarette smoking causes abnormalities in the blood circulation that can lead to cardiovascular disease. Similar effects have been attributed to habitual vaping but the reported findings have been less consistent than for smoking. One can assess this toxic effect on the blood vessels at an early stage by measuring the blood vessels' ability to enlarge when stimulated (endothelial function). This characteristic can be evaluated non-invasively in humans. The vapor from e-cigarettes exposes the airways and lungs to the highest concentration of its toxic constituents. Therefore, the earliest effects of vascular toxicity should be detectable in these circulations. The research team will assess endothelial function in the blood circulation of the airways (bronchi), lung and the systemic circulation outside the lungs in vapers and ex-vapers, compared to smokers and exsmokers. It is expected that the team will also investigate the mechanism whereby vape damages the blood vessel in vitro. It is expected to find that vaping has similar effects on the blood vessels as smoking and that the effect is the greatest in the airway circulation and reversible after cessation of vaping and smoking. Early detection of vaping's toxic effect on the blood circulation can prevent the development of chronic vascular disease.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

James and Esther King Biomedical Research Program
Appendix M
Fiscal Year 2020-2021 Active Grants
Funding Year 2019-2020

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
20K01	Florida State University	Pradeep G. Bhide, PhD	$626,708	5/31/2023	No	Yes	No
20K02	Mayo Clinic Jacksonville	Debabrata Mukhopadhyay, PhD	$626,708	4/30/2023	No	No	No
20K04	University of Central Florida	Ulas Bagci, PhD	$1,112,880	4/30/2023	No	Yes	No
20K05	University of Florida	Terence E. Ryan, PhD	$626,710	5/30/2023	No	No	No
20K06	University of Florida	Gilbert R. Upchurch, Jr., MD	$626,708	5/31/2023	No	No	No
20K07	University of Florida	Daiqing Liao, PhD	$626,708	5/31/2023	Yes	Yes	No
20K08	University of Florida	Dorian K. Rose, PhD, PT	$688,940	5/31/2025	No	No	No
20K09	University of Miami	Ami P. Raval, PhD, MSPH	$626,710	5/31/2023	No	Yes	No
20K10	University of Miami	Taghrid Asfar, MD, MSPH	$1,253,415	5/31/2025	No	No	Yes
20K11	University of Miami	Miguel A. Perez-Pinzon, PhD	$626,708	5/31/2023	No	No	No

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

Principal Investigator: Pradeep G. Bhide, PhD

Organization: Florida State University

Abstract: The goal of this research project is to examine the effects of combustible and e-cigarette exposures on the developing brain, germ cells and future generations using a mouse model. As the project began, COVID-19 restrictions prevented use of equipment in the collaborator's laboratory. Therefore, new equipment was purchased for this project. Although COVID-19 restrictions delayed installation and calibration of the equipment, the studies began in 2021.

The initial studies focused on exposure of mice to combustible cigarette smoke. Adult female mice were exposed to cigarette smoke or room air (control). Each group received two second puffs per minute of cigarette smoke or room air for 48 minutes per day, and three-four days a week. The mice were exposed for three-four weeks prior to breeding with drug naïve males and throughout pregnancy. Upon parturition, the mother and her offspring were exposed for another three weeks. The exposure period corresponds to human exposures beginning prior to conception and continuing through all three trimesters of pregnancy. The health and wellbeing of the mice was monitored daily by visual inspection. In addition, the mice were weighed at the beginning of the exposure as well as twice a week during the exposure period. No adverse health effects were noted in the room air or cigarette smoke exposure groups. In addition, there was no statistically significant difference in the percentage change in body weight between the room air (control) group and the cigarette smoke exposure group. These data suggest that the

cigarette smoke exposure paradigm was not associated with adverse effects on the general wellbeing or body weight gain.

The concentration of cotinine, a major metabolite of nicotine, was measured in the plasma and urine samples of the mice once a week to perform a quantitative assessment of the level of nicotine exposure. As expected, cotinine was not detected in the plasma or urine samples of room air exposed control mice. On the other hand, the mice in the cigarette smoke exposure group showed on average 80ng/ml nicotine in the plasma and urine samples. The plasma cotinine concentration is comparable to that in earlier studies in which nicotine was delivered via drinking water, and it is equivalent to the cotinine concentrations in people who smoke 17-20 cigarettes per day.

In another set of studies, female mice were exposed to e-cigarette aerosol or e-liquid alone. The mice received a two second puff (35-55ml) of e-cigarette aerosol followed by 58 second exposure to fresh air. The system is being calibrated (i.e., wattage of the electric coil) to achieve plasma cotinine levels of 70-80 ng/ml following each session of e-cigarette exposure. As was the case with the combustible cigarette exposure (ongoing), the e-cigarette exposure of female mice will begin three-four weeks before they are bred with drug naïve male mice and continue through pregnancy and the three-week post-partum nursing period.

In summary, a paradigm of combustible and electronic cigarette smoke exposure in a mouse model is being established to study the effects on the developing brain and behavior.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: McCarthy DM, Lowe SE, Morgan TJ, Cannon EN, Biederman J, Spencer TJ, Bhide PG. Transgenerational transmission of behavioral phenotypes produced by exposure of male mice to saccharin and nicotine. Sci Rep. (2020) doi: 10.1038/s41598-020-68883-6.PMCID: PMC7371742.

McCarthy DM, Bhide PG. Heritable consequences of paternal nicotine exposure: From phenomena to mechanisms. Biology of reproduction. (2021). Epub 2021/06/15. doi: 10.1093/biolre/ioab116. PubMed PMID: 34126634.

Zhang L, Levenson CW, Salazar VC, McCarthy DM, Biederman J, Zafonte R, Bhide PG. Repetitive Mild Traumatic Brain Injury in a Perinatal Nicotine Exposure Mouse Model of Attention Deficit Hyperactivity Disorder. Dev Neurosci. (2021) doi: 10.1159/000515198. PubMed PMID: 33849015.

Patents: None at the time of reporting.

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

Principal Investigator: Debabrata Mukhopadhyay, PhD

Organization: Mayo Clinic Jacksonville

Abstract: The primary goal of this project is to develop a novel therapeutic strategy for lung cancer patients especially for any smoking-induced exacerbations of the disease.

A standard operating procedure (SOP) was developed for generating cigarette smoke extract (CSE) in a reproducible manner. In vitro cell proliferation assays were performed in mouse lung cancer cell lines treated with CSE and LNDT-19, a liposomal formulation of NDT-19, a multi-target inhibitor. LNDT-19 showed good antiproliferative effect even in presence of CSE. Western blot experiments showed that CSE increased the expressions of several proteins important for cancer growth and metastasis while LNDT-19 treatment decreased their expressions, suggesting LNDT-19 could inhibit CSE induced signaling pathways. Several new formulations with varying amount of cationic lipids were screened for better tumor targeting in orthotopic lung cancer models. The formulation with 10% cationic lipid showed significantly higher lung homing. Hence NDT-19 liposomes with 10% cationic lipid were prepared that showed good size distribution and polydispersity index, suggesting a homogeneous liposomal formulation. The change in expression of several immunomodulatory cytokines were analyzed due to exposure with CSE or NDT-19. NDT-19 inhibited some tumor-promoting cytokines but increased the expression a pro-inflammatory cytokine as well, suggesting a plausible dual role of NDT-19 in cancer. Orthotopic LLC1 tumors were developed in C57BL/six mice for the tumor growth inhibition study using four treatment groups: i) control, ii) LNDT-19 iii) CSE and iv) CSE + LNDT-19. Unfortunately, due to aggressive tumor growth, the experiment was stopped after one week of treatment and any inhibitory effect of LNDT-19 was not clear at this timeframe. However, CSE treated group showed some increase in tumor growth compared to control. The tumor growth inhibition study was repeated using lower cell number and higher dose of NDT-19. Mice with tumors were randomized into two treatment groups (five mice/group): i) control, ii) NDT-19. Contrary to expectations, NDT-19 increased the tumor growth in this model. A different cell line will now be used to ensure our observations are not a particular cell line specific. Also, new NDT-19 derivatives will be screened for their efficacy in vivo to identify the derivative with good antitumor effect in immune-competent models. Cytokine expressions will be analyzed to ensure no tumor-promoting cytokines are overexpressed due to treatment. A related project was initiated to analyze the efficacy of a new potential NRP1 inhibitor in combination with a known tyrosine kinase inhibitor (TKI) in overriding TKI resistance in NSCLC. After two weeks of treatment, the combination group showed higher tumor growth inhibition than control and monotherapy groups in vivo.

The research is still not at the juncture where it can directly be beneficial to patients as rigorous screening and validation experiments are needed before going for clinical trials, but the results obtained from this study will certainly pave the way for a better strategy in combating lung 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.

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

Principal Investigator: Ulas Bagci, PhD

Organization: University of Central Florida

Abstract: Significant progress has been made with respect to the planned timeline of the project. The overall goal is to develop explainable AI methods for radiation therapy for lung cancer patients and predict any recurrence of the tumors/therapy effects. Tasks include data collections, algorithm development, user-interface development for explainable AI component, decision systems with human in the loop, and numerous experiments with various data sets to validate the algorithms. Weekly meetings have continued (KnowledgeVis and Phd student(s)), biweekly or monthly meetings (all teams together), and continued journal club meetings (weekly) for this project.

The research staff collected both publicly available data sets as well as unique data sets from a collaborating institute, the Orlando Health Cancer Center. The public NSCLC (Non-small Cell Lung Cancer) data set (315 anonymized CTs), LIDC-IDRI (Lung Image Database Consortium) (>1000 CT scans) and head/neck data sets OpenKBP (>40 CT scans) were used from public data sites. From Orlando Health, data has been collected from 98 anonymized radiotherapy patients treated at Orlando Health during 2012, 2016, 2017, and 2018. These images represent good “ground truth” data that can be trusted for use to train deep neural networks (for segmentation and dose prediction). Overall, the team has surpassed timeline goals in collecting data for this project.

Follow on Funding: None at the time of reporting.

Collaborations: Collaboration was with Northwestern University Radiation Oncology Department and Massachusetts General Hospital, Professor G. Sharp.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Terence E. Ryan, PhD

Organization: University of Florida

Abstract: Chronic tobacco smoking remains a major health concern for Floridians. Skeletal muscle atrophy and weakness are commonly reported by patients who are active or former smokers, which contributes to poor quality of life as people age. With this as the framework, the long-range goal of this grant is to provide a basis for developing therapies to reduce muscle atrophy secondary to chronic tobacco smoke exposure, which based on epidemiological data could positively impact nearly half a million people statewide. The purpose of this grant is the examine the role of the aryl hydrocarbon receptor, a protein within the cell that binds many

chemical toxins in tobacco smoke, in the development of muscle atrophy with smoking. While acute activation of this receptor is usually considered adaptive, chronic activation has been shown to be toxic in many cells, however nothing is known about its role in skeletal muscle.

Research staff have established that acute tobacco smoke exposure activates this receptor and have now developed a mouse strain that allows us to delete this receptor only in skeletal muscles and project staff are in the process of exposing these mice to daily tobacco smoke to test if this reduces the amount of muscle loss and weakness. In addition to deleting this receptor, research staff are performing experiments in normal health mice that express a chronically active receptor to mimic tobacco smoke exposure. These sets of experiments are ongoing and being completed in a rolling manner as mice reach a mature age to enroll in these experiments. Experiments from these mouse studies are currently blinded to ensure the highest standards of scientific rigor, so results are unknown at the time of reporting.

To date, the most significant completed results stem from the third Aim of the project which seeks to determine the major chemicals within tobacco smoke that decrease mitochondrial function. Mitochondria are an organelle within the cell are most known for producing energy, but these become impaired with tobacco smoking. Research staff have performed an in-depth chemical analysis of tobacco smoke extracts and have begun screening individual chemicals for a negative impact on mitochondria. At the time of reporting, more than 35 individual chemicals have been screened in mitochondria, with several chemicals identified as negatively impacting mitochondrial energy production. This screening process is continuing with more rounds of individual chemicals identified in the tobacco smoke.

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

Principal Investigator: Gilbert R. Upchurch, Jr., MD

Organization: University of Florida

Abstract: The purpose of this project is to investigate the effect of nicotine on immune cell infiltration and activation of myeloid-derived suppressor cells (MDSCs) via CXCR2 (chemokine receptor type 2) and IL-17 (interleukin-17) signaling that mediates abdominal aortic aneurysm (AAA) formation.

Aortic aneurysms affect 5% of the population aged >65 years, with the incidence three to five times higher in smokers than in nonsmokers. Furthermore, tobacco smoke increases the rate of aortic expansion and the risk of aortic rupture. However, how tobacco use influences aortic aneurysms and rupture has not been addressed. In this proposal, research staff propose that tobacco smoking and age alter epigenetics regulated myeloid derived suppressor cell (MDSC) numbers and function that exacerbate inflammation and vascular remodeling during abdominal

aortic aneurysm (AAA) formation and rupture. To abrogate these critical cytokine dependent inflammatory pathways, the team will analyze if cultured G- and M-MDSCs (with/without nicotine exposure) can significantly upregulate immunosuppress T cell activation and IL-17 secretion. An established murine AAA model of topical elastase-treatment will be used in the present study. The trafficking and migration of MDSCs to the aortic tissue and role of CXC Chemokine receptor 2 (CXCR2) and programmed death-1 (PD-1) receptor signaling was deciphered using an in vivo elastase-treatment model of AAA as well as in vitro co-culture experiments.

The latest data showed that elastase-treatment of wild type (WT) mice resulted in a multi-fold increase in M-MDSCs (CD11b+Ly6G-Ly6C+) in aortic tissue of elastase-treated WT mice on day 14 compared to heat-inactivated elastase controls (15.5±3.3 vs. 0.42±0.13%; mean±S.E; p=0.02; n=4/group).In a separate experiment, elastase-treated mCXCR2-/- mice (myeloid cell-specific deletion of CXCR2) showed a trend towards decrease in M-MDSCs compared to elastase-treated WT mice (6.2±1.1 vs. 12.6±2.7%; p=0.14; n=4-5/group). Furthermore, using the elastase-treatment in vivo model of AAA formation, staff observed that implantation of nicotine pellets (5 days prior to and continued till day 14 post-elastase treatment; 5mg with release rate of 2.2 mg/kg/day) significantly increased abdominal aortic diameter in elastase-treated C57BL/6 (wild- type; WT) mice compared to elastase-treatment alone (1.4±0.05 vs. 1.16±0.07 mm; n=6/group; p=0.02). Previous in vitro data shows that treatment of aortic smooth muscle cells (AoSMCs) with transient elastase treatment (5-minute exposure followed by wash with PBS and replacement of media) induces significant and multi-fold increase in proinflammatory cytokine secretion of IL-6, KC, and MCP-1 (n=6/group; $p<0.05$ vs. controls). Moreover, our in vitro data shows that the secretion of IL- 1b from RAW264.7 macrophages was significantly enhanced by concomitant nicotine treatment (10mM) with transient elastase (0.4U/ml) compared to elastase treatment alone after 24hrs (148±11 vs.91±11 pg/ml; mean±S.E.; n=7/group; $p<0.04$). Statistical evaluation was performed with GraphPad Prism 8 software and values are presented as mean ± SEM. One-way ANOVA or t-tests were used and $p<0.05$ was considered statistically significant.

The impact of nicotine on MDSC recruitment and trafficking to cause macrophage- and smooth muscle cell-dependent inflammation in aortic inflammation is being deciphered by our recent results. These findings also implicate the specific role of MDSC subsets and CXCR2 signaling that has the potential to influence aortic inflammation and vascular remodeling to cause AAA formation.

Follow on Funding: None at the time of reporting.

Collaborations: This research is being conducted in the Aortic Aneurysm Research laboratory at the Department of Surgery, University of Florida, Gainesville, FL. The trainee, Sara Hensley, MD (postdoctoral fellow) is performing research under this project.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Daiqing Liao, PhD

Organization: University of Florida

Abstract: Breast cancer is the most commonly diagnosed cancer type and the second leading cause of cancer-related mortality for women. About one in eight women in the U.S. will develop invasive breast cancer over the course of her lifetime. In 2021, 284,200 new cases of breast cancer are expected to be diagnosed in the U.S. with 20,160 cases in Florida. Advanced breast cancer is still very difficult to treat and the prognosis for metastatic breast cancer remains poor. Therefore, development of new therapy for advanced breast cancer is urgently needed to improve treatment outcome for patients with advanced breast cancer. Increased lipid production in cancer cells promotes cancer cell proliferation and drives cancer progression. The goal of this grant is to determine the mechanisms underlying increased lipid production in breast cancer cells and devise potential therapy to inhibit this process for effective cancer treatment. Thanks to this grant support, significant progress has been made including peer-reviewed publications, training of students at undergraduate and graduate level, and the creation of intellectual properties. Ultimately, the scientific knowledge gained from this project may lead to novel and effective therapies for treating advanced breast cancer.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Waddell AR, Huang H and Liao D. (2021) CBP/p300: Critical Co-activators for Nuclear Steroid Hormone Receptors and Emerging Therapeutic Targets in Prostate and Breast Cancers. (Basel). 13(12):2872. PMCID: PMC8229436 https://pubmed.ncbi.nlm.nih.gov/34201346/

Waddell AR, Ding H, Huo Z and Liao D. (2021) Pharmacological inhibition of CBP/p300 blocks estrogen receptor alpha (ERa) function through suppressing enhancer H3K27 acetylation in luminal breast cancer. Cancers (Basel), 13(11):2799. PMCID: PMC8200112 https://pubmed.ncbi.nlm.nih.gov/34199844/

Xiao Y, Wang J, Zhao LY, Chen X, Zheng G, Zhang X, Liao D (2020). Discovery of histone deacetylase 3 (HDAC3)-specific PROTACs. Chem Comm 56(68):9866-9869. PMID: 32840532, PMCID: PMC7654701 https://pubmed.ncbi.nlm.nih.gov/32840532/

Waddell AR, Liao D (2020) Assays for Validating Histone Acetyltransferase Inhibitors. J. Vis. Exp. 6(162), e61289, doi:10.3791/61289. PMID: 32831305 https://pubmed.ncbi.nlm.nih.gov/32831305/

Patents: Daiqing Liao, Compounds And Methods For Reducing The Amount Of Rest Compressor 1 And/Or Lysine-Specific Histone Demethylase In A Subject,T18443US001 (222107-8725), University Of Florida Research Foundation

Daiqing Liao, Yufeng Xiao, Xuan Zhang, Guangrong Zheng Benzoylhydrazide-Derived Hdac Degraders As Therapeutics For Treating Cancer And Other Human Diseases Ref No.: 222107-2840 (T18184WO001), University Of Florida Research Foundation

Daiqing Liao, William R. Roush, Ryan L Stowe HDAC INHIBITOR COMPOUNDS AND METHODS OF TREATMENT Ref No.: UF#-14839 (222109-1920) (TSRI# 1698.2 US / TSR2049P),University of Florida,The Scripps Research Institute

Daiqing Liao, Iqbal Mahmud, Guimei Tian Polypeptide Inhibitor Of De Novo Lipogenesis In Cancer Cells, T17034US002 (222107-1075), University Of Florida Research Foundation

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

Principal Investigator: Dorian K. Rose, PhD, PT

Organization: University of Florida

Abstract: This randomized controlled trial examines the benefit of the addition of respiratory exercises to a community-based stroke wellness program for Floridians living with the consequences of stroke. The research project aims to enroll 80 individuals over five years. In the first year of this award staff successfully obtained Institutional Review Board approval, hired and trained study personnel and developed and launched recruitment strategies following Notification of Award. The research project team was able to recruit and enroll their first study participant in November 2020. Since then, 14 individuals have enrolled, meeting our first year recruitment goal. Eleven participants have completed the study's intervention phase and are currently in the follow-up phase. There have been no study withdrawals or lost to follow-up. As this is a randomized controlled trial, the research team will not have definitive results until enrollment and all study assessments are complete. Cross-sectional data were presented March 9, 2021 for the Brooks-University of Florida Research Connection event attended by health professionals locally and nationally. Additionally, pre-intervention vs. post-intervention data of the entire cohort (not by group randomization) were submitted for peer review for a 2022 professional meeting. The research team meets bi-weekly to discuss recruitment, intervention and retention and to problem-solve any challenges.

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 #:** 20K09 Nicotine Alters Brain Metabolism and Exacerbates Brain Damage

Principal Investigator: Ami P. Raval, PhD, MSPH

Organization: University of Miami

Abstract: Despite global warnings and awareness of the detrimental effects of smoking on health, smoking-derived nicotine dependence makes relinquishing the smoking habit more difficult for women than for men. Smoking-derived nicotine also makes females more susceptible to ischemic brain damage, a consequence of stroke or cardiac arrest. Both these diseases are responsible for long-term disability or death in women, for which smoking is the one preventable risk factor. Giving up smoking reduces the risk of brain damage from ischemia. Women smokers trying to quit smoking often switch to the electronic nicotine delivery systems (e-Cigarettes). Packaging and websites for e-Cigarettes reveal unsubstantiated health claims and erroneous nicotine content labeling, which along with their wide combination of flavorings and “high-tech” image also make e-cigarettes attractive to youth and young adults. The results of our research demonstrate that nicotine inhibits estrogen-mediated benefits in the brain and makes the brain more susceptible to ischemic damage. Smoking-derived nicotine (N) and oral contraceptives (OC) synergistically magnify cerebral ischemia risk in women. The proposed research aims to validate our findings in animal models and translate these findings to better understand how women’s brains are affected by nicotine. In a lab study using an animal model of cerebral ischemia, staff demonstrates that chronic nicotine exposure renders females more susceptible to ischemic brain damage (d'Adesky et al., 2020). Most importantly, the severity of ischemic brain damage is far greater in females simultaneously exposed to OC than to nicotine-alone (Raval et al., 2012b). In females, estrogen protects the brain from ischemic injury by activation of its receptor subtype (ER-b). The simultaneous exposure to N+OC reduces availability of ER-b at the brain mitochondria has been demonstrated. Our published study shows that activity of Minochondrial Complex IV(CIV) is directly regulated by ER-b (Raval et al., 2012a). CIV activity is compromised if there are any defects in the assembly and⁄or changes in subunit phosphorylation status. This team previously showed that N+OC induces loss of mitochondrial ER-b, and in a previous quarterly report provided data that ER-b activation improves CIV subunit assembly status. In recently published studies staff demonstrated that N+OC alters brain metabolism (d'Adesky et al., 2020; Diaz and Raval, 2021) and are currently performing studies to investigate how long these deleterious effects of N+OC lasts in the brain of female rats.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Huberman MA, d’Adesky ND, Niazi QB, Perez-Pinzon MA, Bramlett HM and Raval AP. Irisin-associated neuroprotective and rehabilitative strategies for stroke. Neuromolecular Med. (2021).

d’Adesky N, Diaz F, Zhao WZ, Bramlett HM, Perez-Pinzon MA, Dave KR, and Raval AP. Nicotine exposure along with oral contraceptive treatment in female rats exacerbates post-cerebral ischemic hypoperfusion potentially via altered histamine metabolism. Transl Stroke Res. (2020).

McCarthy M and Raval AP. The Peri-menopause in a Woman’s Life: A Systemic Inflammatory Phase that Enables Later Neurodegenerative Disease. J Neuroinflammation. (2020).

Schatz M, Saravanan S, d'Adesky ND, Bramlett H, Perez-Pinzon MA, Raval AP. Osteocalcin, ovarian senescence, and brain health. Front Neuroendocrinol. (2020).

Patents: None at the time of reporting.

9. **Grant #:** 20K10 Developing and Testing Waterpipe-Specific Health Warning Labels Targeting Young People in Florida

Principal Investigator: Taghrid Asfar, MD, MSPH

Organization: University of Miami

Abstract: During the first year, the team fully executed the sub-award with Florida International University and the consulting agreements with Golin/Harris and the Truth Initiative. The sub-award with Dr. Schmidt will be completed during the summer of 2021. Monthly meetings are held and include the entire team and consultants to collaborate on all study progress.

During the first year of the study, significant progress towards Aim 1 has been made. The team received IRB approval for the study, and hired and trained two research associates and a statistician for the project. The original target was to develop 12 health warning labels, but the team has doubled the number of labels and added two new themes related to addiction and COVID-19-related risks. Testing of the final 24 labels has begun in the focus group study and eight focus groups have been completed with a total of 21 participants.

The team conducted a preliminary analysis with each focus group to understand which labels appear to be most effective, which labels do not resonate with this population, and which could benefit from modifications to make them more compelling. Participants have overall provided constructive feedback, both positive and negative, and at least one label from each of the six themes has received significant positive feedback. Constructive feedback for improving the labels has also been provided.

Recruitment of eligible participants has been a significant challenge due to COVID-19. In response, the team has initiated an extensive advertising effort to create content to promote the study on Facebook and other social media platforms. Study flyers are distributed at local hookah cafes and restaurants, the University of Miami campus, via student listservs, on the university's Department of Public Health Sciences website, research listserv, and at Metrorail stations in Miami. Recruitment is expected to pick up once students return to campus in the Fall and the situation of COVID-19 improves in Miami.

On parallel to focus groups, an online rating survey has been initiated to obtain quick feedback on the health warning labels. A total of 49 participants have completed the online rating survey. To promote this online rating survey, a contract with Kantar, Inc. has been executed, a data and research services company, to assist with recruitment in the target population throughout the state of Florida. The goal is to recruit 250 eligible waterpipe smokers in the rating study.

The study has been introduced at several statewide tobacco control meetings including Students Working Against Tobacco (SWAT), where waterpipe risk education materials were provided, and the Consortium for a Healthier Miami-Dade Tobacco Free Workgroup.

Two abstracts are being prepared for the 2022 Annual Meeting of the Society for Research on Nicotine and Tobacco (SRNT). The first will focus on analyses from quantitative data collected from the online rating survey of health warning labels. The second abstract will focus on

qualitative data collected during the focus groups as well as quantitative from the surveys of those focus group participants.

The team is in the final stages of creating a website for the project to disseminate knowledge about the harmful effect of waterpipe smoking, publish our health warning labels for others to use and test, and to promote the study and its findings. This site will be launched August 15, 2021 and contains information about the research team, the background and aims of the study, the developed health warning labels, current research findings on the risks of waterpipe smoking, regulations related to waterpipe smoking, and resources for quitting.

Follow on Funding: National Institutes of Health, “Develop and Test Health Warning Labels on Electronic Nicotine Delivery Systems (ENDS).”, Dr. Asfar and Dr. Maziak.

Collaborations: Dr. Wasim Maziak of Florida International University and his research team have been instrumental in the development and review process of the health warning lables as well as efforts to promote the study through statewide meetings and through recruitment efforts at Florida International University.

Dr. Michael Schmidt of the University of Memphis played the key role as public health graphic design expert to generate, adapt and revise the health warning lables for focus group testing. He is actively involved in the analysis of focus group feedback in preparation for the final revisions to be tested in the lab experimental study in Aim 2.

Mr. Ian Abrams of Golin/Harris International has been instrumental in providing opportunities to present information about our study to statewide tobacco control groups, and they have been actively involved in the develoment of the website design and content.

Dr. Donna Vallone from the Truth Initiative has a key role in disseminating our research findings, and promoting our developed health warning labels through the campaign.

During the first year we fully execute the subaward with Florida International University and the consulting agreements with Golin/Harris and the Truth Initiative. The subaward with Dr. Schmidt will be completed during the summer of 2021. We hold monthly meetings that include the entire team and our consultants to collaborate on all study progress.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

10. **Grant #:** 20K11 Strategies to Ameliorate Cognitive Decline Following Cerebral Ischemia in Nicotine-exposed Rats

Principal Investigator: Miguel A. Perez-Pinzon, PhD

Organization: University of Miami

Abstract: As a summary from our prior progress reports: 10 days after transient focal cerebral ischemia animals belonging to sham MCAo + sham exercise, MCAo + sham exercise, and MCAo + exercise (forced treadmill exercise 12 m/min for a total of six days starting day three post-MCAo) were euthanized for histopathology. Tissues were stained with hematoxylin and

eosin (H&E) and neurons with normal were counted. Neuronal density (neurons/mm3) was measured in the septal nucleus, found on sections 0.2, 0.48, and 0.70 mm from bregma. Neuronal density was also quantified among anterior thalamic nucleus found on sections -1.60 and -1.40 mm from bregma.

Following ischemia, histopathology revealed significant differences in neuronal survival in septal nucleus in rats that underwent sham physical exercise post-ischemia compared to sham MCAo + sham exercise. The neuronal density in MCAo + sham exercise group (13,725 ± 638, n = 7) was significantly lower by 52% compared to sham MCAo + sham exercise group (28,826 ± 2,424, n = 8). The neuronal density was higher by 37% in MCAo + exercise group (18,765 ± 869, n = 7) compared to MCAo + sham exercise group. The neuronal density in MCAo + exercise group remained lower by 35% compared to sham MCAo + exercise group. However, these differences were not statistically significant. For anterior thalamic nucleus, the neuronal density in sham MCAo + sham exercise, MCAo + sham exercise, and MCAo + exercise groups were 12,144 ± 1,108 (n = 9), 10,814 ± 910 (n = 10), and 10,369 ± 737 (n = 6), respectively.

The research team has effectively developed staining of the septal nuclei with glutamate acid decarboxylase 67 (GAD67) to identify whether the cells affected in this important nucleus, are GABAergic cells. Successful development of the immunohistochemistry using choline acetyltransferase (polyclonal-ChAT) to determine if MCAo affects the number of cholinergic cells in these nuclei. Staff have not finished quantifying the number of surviving cell populations in these different nuclei, because these studies are quite extensive. However, the team has determined the number of GAD67 and ChAT positive cells in two group of animals, sham MCAo and MCAo (n=6) allowed to survive 22 days, just like in our previous studies. Preliminary studies revealed significant declines in the number of GAD67 positive cells (GABAergic) in the lateral and medial septum in the ipsilateral compared to contralateral and compared to shams. No differences were observed in ChAT positive cells (cholinergic). At the level of the diagonal band, only the horizonal band exhibited significant declines in the number of both GAD67 and ChAT positive cells in the ipsilateral side compared to contralateral and sham MCAo. Both septal and diagonal band nuclei are crucial limbic system nuclei regulating theta oscillations in the hippocampus and other brain regions that are crucial for memory encoding and retrieval.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

James and Esther King Biomedical Research Program
Appendix N
Fiscal Year 2020-2021 Active Grants
Funding Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
9JK01	Florida State University	Gloria Salazar, PhD	$1,409,467	9/30/2022	No	No	No
9JK02	H. Lee Moffitt Cancer Center	Shelley S. Tworoger, PhD	$504,838	8/31/2022	No	Yes	No
9JK03	Miami Cancer Institute, Baptist Health South Florida	John P. Diaz, MD	$1,187,224	12/31/2024	No	No	No
9JK04	University of Central Florida	Alicja J. Copik, PhD	$805,409	9/30/2022	No	Yes	No
9JK05	University of Florida	Ramzi G. Salloum, PhD	$404,909	9/30/2022	No	Yes	No
9JK06	University of Florida	Maria Zajac-Kaye, PhD	$805,409	9/30/2022	No	No	No
9JK07	University of Miami	Sundaram Ramakrishnan, PhD	$805,393	8/31/2022	No	No	No
9JK08	University of Miami	Kunjan R. Dave, PhD	$805,409	9/30/2022	No	Yes	No
9JK09	University of Miami	Ashok Saluja, PhD	$805,409	09/30/2022	No	No	No
9JK10	University of South Florida	Rex M. Philpot, PhD	$771,341	7/31/2022	No	No	No

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

Principal Investigator: Gloria Salazar, PhD

Organization: Florida State University

Abstract: Smoking and aging are two major risk factors for cancer and cardiovascular disease (CVD). Although recent reports showed that smoking stimulates senescence (aging) in the lung, it is unknown whether smoking also accelerates senescence of the cardiovascular system. Research staff hypothesize that aging and smoking activate a common molecular mechanism that depends in part on the NADPH oxidase Nox1 (an enzyme that produces reactive oxygen species) and activation of the senescence associated secretory phenotype (SASP), a process by which senescent cells modify the microenvironment inducing inflammation and tissue dysfunction. The team has demonstrated that polyphenols isolated from blackberries reduce oxidative stress and senescence induced by angiotensin II (Ang II), a strong stimulator of senescence and CVD, by inhibiting Nox1 in vascular smooth muscle cells (VSMCs). This proposal will test the hypothesis that blackberry polyphenols target the Nox1 pathway to reduce reactive oxygen species (ROS) levels and activation of the SASP, thus diminishing senescence and atherosclerosis caused by tobacco smoke and nicotine. For this reporting period, the team assessed the molecular mechanism by which cigarette smoke and nicotine induce senescence and atherosclerosis in male and female mice (aim 1). It was found that female ApoE knock out

mice exposed to cigarette smoke accumulate more plaque and senescence compared with nicotine and with males. In contrast, males exposed to cigarette smoke and nicotine showed similar levels of plaque and senescence suggesting that nicotine elicit the same detrimental effects of cigarette smoke. The higher level of plaque in females correlated with an increased level of pro-inflammatory cytokines in circulation. Control females had higher levels of interleukin 17A (IL-17A), IL-1alpha and the lipopolysaccharide-induced CXC chemokine (LIX). Cigarette smoke and nicotine increased IL-17A in both sexes, while cigarette smoke increased LIX only in males and IL-1alpha only in females. Thus, our data show sex-dependent responses to cigarette smoke and nicotine for plaque and expression of inflammatory markers. These data highlight the importance of using both males and females to assess the effects of tobacco products and the detrimental effects of nicotine in the cardiovascular system. For aim 2, cigarette smoke extracts (CSE) and nicotine were used to assess senescence and oxidative stress in VSMCs in culture. Although not proposed in the original proposal, the team used VSMCs that were isolated from the aortas of male and female mice. Similar to in vivo experiments, female VSMCs were more sensitive to CSE and showed a higher basal senescence compared with male cells. Interestingly, it was found that male VSMCs display a protective mechanism when exposed to CSE that was not observed in female cells. This mechanism depends on the expression of sequestosome 1 (SQSTM1), an autophagy receptor. Sex-dependent effects for IL-1alpha, IL-17A and LIX in cells in culture were also found. Overall, significant advances in aims 1 and 2 have been made, and the team plans to start working on aim 3 this fall. A manuscript is being prepared that will be submitted to Cell Metabolism (Impact factor 21.5) in which the sex-dependent effects of cigarette smoke and nicotine will be reported.

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 #:** 9JK02 Early Life Exposures and Risk of Developing Ovarian Cancer

Principal Investigator: Shelley S. Tworoger, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: Ovarian cancer is the deadliest gynecological cancer in Florida and is responsible for nearly 1,000 deaths annually statewide. Increasing evidence suggest that childhood and adolescence are critical periods when exposures can alter how the ovaries develop and impact the likelihood of ovarian cancer development in adulthood. During the current reporting period, the research staff conducted analyses of early smoking exposure, physical activity, abuse and social economic status in relation to risk of ovarian cancer using data from two large studies of U.S. women that have been followed by biennial questionnaires since 1976. A manuscript describing findings of early life smoking and ovarian cancer risk was accepted for publication by the International Journal of Epidemiology (IJE) on January 21, 2021. Moreover, the study team's

finding on early life smoking was selected to be presented both orally and as a poster for the 45th annual meeting of the American Society of Preventive Oncology (ASPO).

In analyses of early life physical activity and risk of ovarian cancer, neither physical activity at ages 12-13, 14-17, or 18-22 years nor average physical activity across these three periods was related to ovarian cancer risk in adulthood. Also, these associations did not differ by early life body mass index (BMI), subtype of ovarian cancer, or menopausal status at cancer diagnosis. These results suggested that early life physical activity was not clearly related to adult ovarian cancer risk. A manuscript describing these findings was submitted to the International Journal of Cancer (IJC) on May 6, 2021 and is under review.

In analyses of early life economic status, the research staff did not observe associations of ovarian cancer risk with early life social economic status. However, mother's age ≥25 at nurses' birth was related to increased risk of ovarian cancer. Together, these findings indicated that mother's age at birth, but not early life social economic status, may have an impact on adult ovarian cancer risk. A manuscript describing these results are in preparation.

For analyses of early life abuse and risk of ovarian cancer, the study team decided that the findings might be clearer, and thus more impactful, with a larger sample size. Colleagues from the Sisters Study finished analyses and sent results back to the study team. No associations of ovarian cancer risk with early life sexual or physical abuse were observed, which were consistent with our results in Nurses' Health Study II. A meta-analysis will be conducted once colleagues from the Black Women's Health Study complete analyses.

Disseminating these results will contribute to justification for targeted messages to parents to avoid smoking inside the home as well as initiatives to prevent abuse, as both of these actions may help decrease incidence of ovarian cancer. In addition, these results may help researchers improve prediction models for ovarian cancer, which would have direct implications for health care delivery and be a substantial benefit to Floridians given the significant burden of ovarian cancer in Florida.

Follow on Funding: None at the time of reporting.

Collaborations: University of Florida: Co-investigator Danielle Jake-Schoffman, PhD is an Assistant Professor in the Department of Health Education and Behavior in the College of Health & Human Performance at the University of Florida, Gainesville, Florida.

Journals: Tianyi Wang, Mary K Townsend, Christine Vinci, Danielle E Jake-Schoffman, Shelley S Tworoger, Early life exposure to tobacco smoke and ovarian cancer risk in adulthood, International Journal of Epidemiology, (2021). https://doi.org/10.1093/ije/dyab018. PMID: 33647961.

Patents: None at the time of reporting.

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

Principal Investigator: John P. Diaz, MD

Organization: Miami Cancer Institute, Baptist Health South Florida

Abstract: Phase II trial, evaluating immunotherapy in combination with PARP Inhibition in advanced cervical cancer patients with functionally competent or deficient for the Fanconi Anemia repair pathway, is currently underway at the Miami Cancer Institute. The COVID-19 pandemic had an unforeseeable impact on the clinical trials program, including this trial. The pandemic resulted in a delay in trial activation and our ability to adequately recruit patients. However, despite these initial setbacks, the team has enrolled three patients on the trial. Additionally, tissue was obtained for evaluation of a targeted marker through our collaboration with Dr. Duan's laboratory at Florida International University.

The trial was presented at the Annual Meeting of the American Society of Clinical Oncology for a virtual presentation in June 2021.

The research team continues to explore strategies to increase enrollment, including expanding clinical trial sites within our health system. Staff are marketing to referring physicians as well as directly to patients through various marketing platforms, including social media. The team is also exploring collaborations with other investigators within the state of Florida. However, these centers have similarly experienced limitations in their clinical trials program due to the impact of the pandemic. Initial discussions with the Promise Fund to increase the patient pool has taken place.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Alicja J. Copik, PhD

Organization: University of Central Florida

Abstract: Non-small cell lung carcinoma makes up 85% of all lung cancer cases and is the leading cause of cancer-related death. Although immunotherapy with checkpoint inhibitors has been a breakthrough for patients with advanced stage lung cancer, the response rate is still low and many patients eventually relapse. The goal of this project is to develop clinically translatable immunotherapeutic strategies for lung cancer treatment to increase the response rate to the

approved checkpoint inhibitor therapies and to lower relapse rate. To achieve the proposed goals, the project is leveraging the unique capabilities of natural killer (NK) cells multiplied to great numbers in our laboratory and reprogrammed to be highly activated through exposure to cellular membrane particles (PM21) or exosomes (EX21) derived from interleukin 21 (IL21) expressing feeder cells (K562-mbIL21-41 bbl, mb21FCs). These cells can be further edited to improve their function and thus potential anti-tumor efficacy which is part of the current work. These PM21-particle stimulated NK cells produce IFNgamma in response to encounters with tumor cells which makes tumor cells induce PD-L1. Presence of PD-L1 has been used as a marker for better outcome of treatment with checkpoint inhibitors such as for example Opdivo. NK cells are also known to recruit other components of the immune system, such as for example T cells to further direct complete elimination of cancer. The team has published a review article that describes the current evidence suggesting that combining infusions of laboratory-grown NK cells with other immunotherapeutic approaches has the potential to improve outcomes and patient survival.

To enhance the function and thus potential clinical efficacy of PM21-NK cell the inhibitory receptors that can put break on NK cell killing need to be turned down. In the past year, the research team made and tested a new version of edited NK cells that now lacks a receptor called T-cell immunoreceptor with IG and ITIM domains (TIGIT) that were found to downregulate PM21-NK cell function.

These edited NK TIGIT knock out (KO) cells kill tumor faster and at much lower concentration. This means that the could control tumor better and less cells would be required to treat patients. The new results have been submitted as a patent disclosure to UCF Technology Transfer office and will be submitted as provisional patent soon. The patents if granted can be licensed and treatments developed in the future by pharmaceutical companies. The PM21-particle method of growing NK cells that this group has developed has been patented and licensed to Kiadis, a Sanofi company together with multiple other patents and patent applications developed by this group. If approved these new treatments will benefit the health of Floridians.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Shaver KA, Croom-Perez TJ, Copik AJ. Natural Killer Cells: The Linchpin for Successful Cancer Immunotherapy. Front Immunol. 2021;12:679117. doi: 10.3389/fimmu.2021.679117. eCollection (2021). Review. PubMed PMID: 33995422; PubMed Central PMCID: PMC8115550.

Patents: None at the time of reporting.

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

Principal Investigator: Ramzi G. Salloum, PhD

Organization: University of Florida

Abstract: To facilitate patient enrollment and data collection for the trial, research assistants were deployed at participating University of Florida Health clinics to assist patients with MyChart activation. Primary delivery of the intervention in the trial will occur via MyChart. The status of MyChart activations is as follows: at CMS Schiebler, portal activation has increased from 39% (June 2020) to 41% (June 2021) among patients 12-17 years old, and from 37% (June 2020) to 46% (June 2021) among patients 0-11 years old; at Magnolia Parke, portal activation has increased from 37% (June 2020) to 47% (June 2021) among patients 12-17 years old, and from 56% (June 2020) to 66% (June 2021) among patients 0-11 years old; at Tower Square, portal activation has increased from 32% (June 2020) to 45% (June 2021) among patients 12-17 years old, and from 45% (June 2020) to 59% (June 2021) among patients 0-11 years old; at Tioga, portal activation has increased from 43% (June 2020) to 57% (June 2021) among patients 12-17 years old, and from 58% (June 2020) to 77% (June 2021) among patients 0-11 years old; questionnaires for all age groups are now in production. We have enrolled a total of 47 participants with tobacco use exposure and 18 participants have completed the three-month follow up questionnaire.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Lee J, Tan AS, Porter L, Young-Wolff KC, Carter-Harris L, Salloum RG. Association Between Social Media Use and Vaping Among Florida Adolescents, (2019). Preventing Chronic Disease. 2021;18.

Patents: None at the time of reporting.

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

Principal Investigator: Maria Zajac-Kaye, PhD

Organization: University of Florida

Abstract: This year´s goal was to continue the antitumoral and survival studies started in the previous year to test novel drug compounds to inhibit thymidylate synthase (TS) for treatment of pancreatic cancer. The laboratory demonstrated that pancreatic overexpression of TS (essential enzyme for DNA synthesis and repair aberrantly overexpressed in a range of human cancers) promoted aggressive Pancreatic Ductal Adenocarcinoma (PDAC) development and markedly reduced survival of genetically engineered (GEMM) Kirsten rat sarcoma viral oncogene homolog (KRAS) mutant mice. Thus, the goal of this proposal is to develop new treatments for pancreatic cancer using unique TS inhibitors identified in our laboratory. Since our preclinical data show that TS inhibitors synergistically enhance RAS/PI3K/AKT/mTOR inhibition in vitro, we proposed in this project to test new TS inhibitors alone or in combination with mTOR inhibitors using our novel hTS/Kras and hTS/Kras. Phosphatase and TENsin homolog deleted on chromosome 10 (Pten) PDAC GEMM models and patient derived xenografts (PDX).

In the past year treatment of hTS/Kras PDAC GEMM was continued to determine the antitumoral effect of compound P, Everolimus and the combination of both. These studies are still in progress due to COVID-19 delay in animal breeding and a long-life span of our GEMMs. We continued enrolling more animals to finish the study in approximately six months. Although it was not observed that mice dying from drug treatment, preliminary pathological analysis of liver tissues from animals treated with compound P alone and in combination with Everolimus suggests that 200 MPK compound P and 5 MPK Everolimus induced hepatic toxicity. Therefore, it is planned to initiate treatment with lower drug concentration. In addition, treatment was started using hTS/Kras.Pten GEMMs and we observed that compound P treated animals live longer compared to untreated controls. We also observed that Everolimus alone was more potent in reducing tumor growth as compared to 200 MPK compound P alone or untreated controls, suggesting that 5 MPK Everolimus may be too high to achieve synergy with 200 MPK compound P. The, it was determined that lower doses of Everolimus induce a potent antitumoral effect and plan to use lower doses of compound P and Everolimus to achieve synergy in vivo.

It was also established a new PDX from a male smoker PDAC biopsy and observed similarly to our results in hTS/Kras.Pten GEMMs that compound P alone resulted in a potent antitumoral effect as compared to untreated controls. When compound P was combined with Everolimus to treat PDX, tumor growth was further reduced; however unexpectedly, treatment with 5 MPK of Everolimus alone further reduced tumor growth as compared to 200 MPK compound P alone or untreated controls. Therefore, as in the GEMM, it is planned to use a lower concentration of compound P and Everolimus to achieve synergy and a potent antitumoral effect, without toxicity in vivo. In addition, we determined that Temsirolimus and TAK-228 (two mTOR inhibitors) show synergy when combined with compound P in vitro and we plan to test their effect in vivo alone and combined with compound P. The final goal of this grant is to lay the groundwork for a personalized investigator-initiated clinical trial at the University of Florida that will reduce PDAC mortality in the Floridian population. Better understanding of this targeted drug combination will enable to treat PDAC patients, improve quality of life and clinical outcomes.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Sundaram Ramakrishnan, PhD

Organization: University of Miami

Abstract: The major focus of the grant was to investigate the role of gut microbiome in cancer growth and progression. In the last quarter research staff have investigated the impact of a major inflammatory mediator, interleukin (IL)-23, in the growth of pancreatic cancer orthotopic

model. IL-23 is upregulated in smoking individuals and cigarette smoke extracts are shown to upregulate IL-23 in animal models and in tissue culture studies. Furthermore, IL-23 levels are higher in pancreatic cancer patients with a history of pancreatic cancer. This anecdotal evidence suggests a potential role for IL-23 in tumor progression. IL-23 is secreted by activated antigen-presenting cells and has been shown to influence differentially the immune microenvironment of the tumors. IL-23 is shown to promote tumor growth. Genetic deletion of IL-23 protects mice from developing chemical carcinogen induced tumorigenesis. Similarly, DMBA-induced skin carcinogenesis was also attenuated in mice lacking IL-23. Therefore, the team evaluated whether neutralization of IL-23 by a monoclonal antibody would slow the growth of pancreatic tumors in a model system. A mouse model of pancreatic cancer was used to evaluate the effect of IL-23 antibody. The studies show that anti-IL23 antibody treatment did not inhibit the growth and metastasis of mouse pancreatic cancer in orthotopic preclinical model. In the future studies, there are plans to investigate the effect of gut microbiome depletion and anti-IL-23 antibodies could potentiate the efficacy of immunotherapy against checkpoint inhibitor, anti-PD-L1 antibody. These investigations will be superimposed with cigarette smoke components on pancreatic cancer growth and metastasis.

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 #:** 9JK08 Nicotine Exposure and Intracerebral Hemorrhage

Principal Investigator: Kunjan R. Dave, PhD

Organization: University of Miami

Abstract: Smoking is one of the main risk factors for spontaneous intracerebral hemorrhage (sICH), the deadliest subtype of stroke. Despite being the cause of significant morbidity and mortality, sICH remains the least treatable stroke subtype. Continued cerebral bleeding leading to hematoma expansion is highest in the first three hours after symptom onset and may continue in many patients between three and twenty-four hours after onset. Hematoma volume in sICH patients correlates with the 30-day mortality rate. Currently, there is no proven therapy to prevent hematoma expansion in sICH patients, and thus clinicians are unable to offer more than supportive care. Several epidemiological studies demonstrated the deleterious effects of smoking / tobacco use in sICH patients. These effects include increased risk of sICH, larger hematoma expansion, and poor post- sICH outcomes. Despite several clinical studies indicating the deleterious effects of smoking / tobacco use in sICH patients, the field is lacking confirmatory systematic preclinical studies evaluating the effects of smoking on outcomes following sICH. The main goal of the proposal is to achieve the goals of the James and Esther King Biomedical Research Program by improving the health of Floridians. In this project, the team proposed to test the hypothesis that chronic nicotine exposure will worsen outcomes

following sICH and red blood cell microparticles (RMP: hemostatic agent) will be able to limit hematoma growth in a clinically relevant animal model of sICH. This project proposed to test this hypothesis by determining the effect of chronic nicotine exposure on outcomes following sICH, the mechanisms by which chronic nicotine exposure increases hematoma volume post-sICH, and if RMP treatment improves post-sICH outcomes in chronic nicotine-treated rats via limiting hematoma growth under the last translational aim. In the recent four quarters of the project, the following accomplishments were made: the team confirmed that chronic nicotine treatment in young female rats resulted in larger hematoma volumes and worsened behavioral outcomes following collagenase-induced sICH; it was established that treatment with red cell microparticles lower hematoma growth in young male and female rats following collagenase-induced sICH; experiments aimed to evaluate the maximum therapeutic window of RMP in preventing hematoma growth following collagenase-induced sICH revealed that RMP therapy is able to limit hematoma growth as well as lower neurological deficits in chronically nicotine-treated young male rats when RMP were administered up to 4.5 h post-sICH induction; it was observed that chronic nicotine treatment in young rats of both sexes and aged male rats resulted in increased reactive oxygen species production in brain vessels; a number of observations were added for experiments that were performed earlier and / or evaluated additional markers to evaluate the impact of chronic nicotine treatment on levels of biomarkers of blood-brain barrier (BBB) integrity; an increased BBB permeability in chronic nicotine-treated aged male rats was observed; and treatment with a TNF-alpha inhibitor reduced hematoma growth in chronic nicotine-treated young male rats following collagenase-induced sICH, no proven therapy to prevent hematoma expansion in sICH patients, and thus clinicians are unable to offer more than supportive care. Several epidemiological studies demonstrated the deleterious effects of smoking / tobacco use in sICH patients. These effects include increased risk of sICH, larger hematoma expansion, and poor post- sICH outcomes. Despite several clinical studies indicating the deleterious effects of smoking/tobacco use in sICH patients, the field is lacking confirmatory systematic preclinical studies evaluating the effects of smoking on outcomes following sICH. The main goal of the proposal is to achieve the goals of the James and Esther King Biomedical Research Program by improving the health of Floridians. In this project, we proposed to test the hypothesis that chronic nicotine exposure will worsen outcomes following sICH and red blood cell microparticles (RMP: hemostatic agent) will be able to limit hematoma growth in a clinically relevant animal model of sICH. We proposed to test this hypothesis by determining the effect of chronic nicotine exposure on outcomes following sICH, the mechanisms by which chronic nicotine exposure increases hematoma volume post-sICH, and if RMP treatment improves post-sICH outcomes in chronic nicotine-treated rats via limiting hematoma growth under the last translational aim. In the recent four quarters of the project, we made the following accomplishments: we confirmed that chronic nicotine treatment in young female rats resulted in larger hematoma volumes and worsened behavioral outcomes following collagenase-induced sICH; we established that treatment with red cell microparticles lower hematoma growth in young male and female rats following collegenase-induced sICH; experiments aimed to evaluate the maximum therapeutic window of RMP in preventing hematoma growh following collagenase-induced sICH revealed that RMP therapy is able to limit hematoma growth as well as lower neurological deficits in chronically nicotine-treated young

male rats when RMP were administered up to 4.5 hours post-sICH induction; it was observed that chronic nicotine treatment in young rats of both sexes and aged male rats resulted in increased reactive oxygen species production in brain vessels; a number of observations were added for experiments performed earlier and/or evaluated additional markers to evaluate the impact of chronic nicotine treatment on levels of biomarkers of blood-brain barrier (BBB) integrity; it was observed increased BBB permeability in chronic nicotine-treated aged male rats; and treatment with a TNF-alpha inhibitor reduced hematoma growth in chronic nicotine-treated young male rats following collagenase-induced sICH.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Cho S, Rehni AK, Dave KR. Tobacco Use: A Major Risk Factor of Intracerebral Hemorrhage. J Stroke. (2021). doi: 10.5853/jos.2020.04770. PMID: 33600701

d'Adesky N, Diaz F, Zhao W, Bramlett HM, Perez-Pinzon MA, Dave KR, Raval AP. Nicotine Exposure Along with Oral Contraceptive Treatment in Female Rats Exacerbates Post-cerebral Ischemic Hypoperfusion Potentially via Altered Histamine Metabolism. Transl Stroke Res. (2020). doi: 10.1007/s12975-020- 00854-5. PMID: 33130995 (In press)

Patents: None at the time of reporting.

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

Principal Investigator: Ashok Saluja, PhD

Organization: University of Miami

Abstract: Tobacco smoking is one of the major risk factors contributing to the development of pancreatic cancer, an aggressive malignancy that has dismal survival rates (less than 10%). Pancreatic cancer is characterized by a complex tumor microenvironment due to the presence of a dense fibro-inflammatory stroma consisting of the extracellular matrix, stromal cells and the infiltrated immune population which makes it resistant to therapy, extremely metastatic and prone to recurrence. Studies from our group as well as others have shown that increased expression of membrane protein CD133 contributes to aggressive biology in pancreatic cancer. These cells are treatment refractory, extremely metastatic and contribute to tumor recurrence. Additionally, CD133 cancer cells undergo dynamic interconversion between aggressive and nonaggressive states by interacting with other cells in the microenvironment. Thus, understanding the molecular mechanism of this dynamic inter-conversion is essential for developing successful therapy against pancreatic cancer. Previously published research from our lab has shown that there is a distinct enrichment of CD133+ cells in the pancreatic tumor stroma which is due to the secretion of stromal IL6 cytokine secretion & signaling. Secreted IL6 also contributes to an altered metabolic phenotype in the CD133+ cells that is responsible for their survival advantage and aggressive phenotype. Based on these observations, the research

team hypothesizes that the stromal component of the microenvironment promotes aggressive biology and metabolic reprogramming in a population of tumor cells resulting in a resistant phenotype. This metabolically rewired cells also lead to an immune suppressive microenvironment, thereby resulting in a tumor that is unresponsive to most therapy. Thus, the team proposes that targeting the stromal secretion will inhibit this plasticity and overcome therapeutic resistance pancreatic cancer. Following are the specific aims that will assist in validating the hypothesis: evaluating the role of stromal component in inducing metabolic reprogramming in pancreatic cancer; elucidating the mechanism by which metabolic reprograming leads to a survival advantage in CD133+ population in PDAC; and whether the stromal secretion can be targeted to overcome plasticity and therapeutic resistance in pancreatic cancer.

So far, results in the above-mentioned grant period show that stroma mediated IL6 promotes tumor “initiation” and enrichment of CD133+ population in pancreatic cancer. Further analysis revealed that secretion of stromal IL6 increases glucose uptake and flux through glycolysis in enriched CD133 + cells and prevents glucose from entering oxidative phosphorylation. By analyzing the IL6 expression in the pancreatic cancer patient population, it was observed that high IL6 expression in pancreatic cancer patients correlated with poor survival. Inhibition of the IL6-IL6 receptor interaction by using IL6 receptor antibody inhibits pancreatic tumor progression in mouse model of pancreatic cancer by increasing CD8+ T cell infiltration but also sensitize pancreatic tumors to immune checkpoint blockade therapy.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Rex M. Philpot, PhD

Organization: University of South Florida

Abstract: It should be noted that due to the COVID-19 crisis, work on the University campus was restricted during much of 2020 and into 2021, until vaccinations became available to most personnel. Nevertheless, during fiscal year two research staff were able to continue, in a limited fashion, assessing chemotherapy-induced cognitive deficits in our mouse model, determining the benefits of muscarinic agonist treatment and collecting key samples for assessment of hormones, neuroinflammation and Central Nervous System (CNS) damage or functional alteration. The most critical research successes from year two are outlined below:

Using mice that reliably develop breast cancer approximately 120 days after birth, the team demonstrated that two drugs used to treat breast cancer under study (Cyclophosphamide and Doxorubicin) interfere with spatial and working memory in mice with cancer. The team also demonstrated that cancer is not necessary for the chemotherapeutic agents to cause problems with learning and memory, but that any issues with learning and memory may be worse when cancer is present. Thus, the team has developed an effective animal model of chemo-brain for these studies.

Data will not be analyzed for statistical significance until all the measurements from all animals have been collected, allowing for appropriate consideration of possible violations of parametric assumptions for statistical tests, ethical removal of outliers and data normalization, and accounting for possible covariates. Thus, the following statements represent observations of tendencies in the data collected thusfar. It was determined that the experimental drugs being tested (Xanomeline Oxylate and VU 0357017) to prevent these problems with learning and memory have some effectiveness at reducing the deficits in spatial and working memory cause by chemotherapy and that these drugs do not: cause the tumors to grow faster; cause an increase in the number of tumors; or interfere with the effectiveness of the cancer treatment drugs, cyclophosphamide and doxorubicin. Therefore, Xanomeline Oxylate and VU 0357017 appear safe to use during cancer treatment for the prevention of chemo-brain.

Staff have determined that cyclophosphamide and doxorubicin administration reduces circulating estradiol and circulating progesterone. The former was predicted based on our hypothesis that estradiol is essential for normal acetylcholine synthesis and that lowered available estrogen will impair normal cognitive function. The impact of the observed progesterone reductions is being examined and is important because progesterone concentrations affect the number of neurons in the brain that produce chemicals involved in learning and memory. This is currently under examination using studies with ovariectomized mice receiving hormone supplementation.

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

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

James and Esther King Biomedical Research Program
Appendix O
Fiscal Year 2020-2021 Active Grants
Funding Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
8JK03	H. Lee Moffitt Cancer Center	Nagi B Kumar, PhD	$708,044	3/31/2023	No	No	No
8JK04	University of Florida	Fredric J. Kaye, MD	$1,360,857	3/31/2023	No	No	No

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

   Principal Investigator: Nagi B. Kumar, PhD

   Organization: H. Lee Moffitt Cancer Center

   Abstract: Although screening high risk populations using low dose Computed Tomography (LDCT) and smoking cessation programs are critical, former smokers on surveillance are eager to participate in chemoprevention interventions that can further reduce their risk for lung cancer. This research team and others have shown that curcumin (CUR) and omega 3 fatty acids (ω-3 FA) are effective at suppressing Stat3P and NF-κB signaling pathways that are relevant to lung carcinogenesis-resulting in suppression of proliferation of human lung tumor lines and inflammation responses. More recently, strong evidence has emerged demonstrating the role and mechanism of ω-3 FA as specialized fat mediators, with anti-inflammatory, anti-proliferative and pro-resolving properties towards resolution of cigarette smoke-induced lung inflammation in former smokers. This team and others have also shown that CUR when combined with ω-3 FA is bioavailable in the lung and produces a more robust antiproliferative effect in lung tumor tissue compared to when these agents administered independently. Based on this evidence, it was hypothesized that a standardized formulation of CUR + ω-3 FA will target molecular pathways that are critical for lung cancers development, leading to a reduction in the overall size and density of nodules, in former smokers. This team hypothesizes that this will be mediated by reducing inflammation and through pro-resolving effects in the nodules. Testing the hypothesis will be done using an experimental design and rigorously evaluating the safety, efficacy and the potential mechanism of a combination of ω-3 FA + CUR or placebo administered for six months in former smokers, with lung nodules detected during LDCT screening program. Results of the proposed trial may have significant benefit to former smokers and other high-risk populations in the state of Florida towards lung cancer prevention.

   The study opened in September 2020 and has continued to screen and recruit subjects to the trial. However, patients were not comfortable coming for this clinical trial until January 2021 (vaccinations available). Although recruitment to this trial has improved, this patient population has the highest risk to relapse and start smoking at screening, although they had originally

claimed to be non-smoker. To improve subject recruitment in this trial, the team will revise the protocol to now include former and current smokers with lung nodules as defined in the inclusion criteria in this trial. Based on the existing evidence in the literature that has been discussed in this protocol, there are no contraindication for use of curcumin in this patient population (current and former). A revised version to include current smokers has been submitted to the Scientific Review Committee of the Moffitt Cancer Center on 4/14/2021. The Scientific Review Board approved this revision on 5/20/2021 (Version 6.0). This was then submitted to the IRB and approved on 6/25/2021. The FDA IND was also approved for the study with these changes. There are 78 current and former smokers are now being interviewed for randomization to trial.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Fredric J. Kaye, MD

Organization: University of Florida

Abstract: Small cell lung cancer (SCLC) is the most lethal subtype of lung cancer as well as the subtype most strongly linked to tobacco exposure. Over the past decade, therapies targeted at specific cancer gene mutations and new immunotherapy strategies have improved the outcome for patients with non-SCLC subtypes. In contrast, there has been a lack of improvement in survival for patients with SCLC over the past decades and Congress specifically emphasized a search for new therapies for SCLC in the Recalcitrant Cancer Research Act H.R.733. Our laboratory has focused on studying why SCLC tumors do not induce host immune cell responses resulting in only a modest benefit from current immunotherapy regimens. In 2019 the team published initial data supporting the delivery of a modified myxomaviral agent (MYXV) to stimulate the immune system, to induce tumor specific cell death, and improve the outcome in SCLC (Oncolytic virotherapy for small-cell lung cancer induces immune infiltration and prolongs survival. J Clin Invest 2019;129(6):2167-2595). The goal of this current project is to translate this pre-clinical data into a novel phase 1 clinical trial testing the safety and efficacy of MYXV injected directly into lung tumor nodules of patients with advanced SCLC.

In the past year, the team submitted a pre-Investigational New Drug (IND) document to the FDA which represents the first step in finalizing approval for a phase 1 clinical trial. Written comments were received from the FDA external reviewers where they had no major concerns with the design and rationale of our proposed clinical study. The FDA gave us specific suggestions

regarding the preclinical animal safety testing which needs to be completed with the Good Manufacturing Product (GMP) clinical material that would be used in the patient studies. 2020 was a challenging year due to the COVID-19 pandemic that resulted in restrictions on research activity. This included the closing of the University of Florida GMP Facility and Vector Core Lab of the UF Powell Gene Therapy Center. As a result, all work on Myxomaviral (MYXV) production was terminated and when the University of Florida Laboratory Facilities re-opened the team had to wait for other priority backlogs within the UF Powell Facility Center. Over the past months, the team has confirmed the MYXV production process for generating clinical grade material using FDA-approved human cell bank and will proceed with completing the animal safety testing required for final FDA approval. Additional preclinical data has been analyzed that further supports the rationale for this clinical trial in patients with advanced SCLC. Over the past year, the team observed that human and murine SCLC tumor samples lack expression of an essential biomarker for host immune cell recognition, designated HLA/MHC1 expression, and has developed strategies to enhance the effectiveness of immunotherapy combined with MYXV delivery. This project will allow testing of an innovative treatment strategy that takes advantage of unique expertise at the University of Florida that has pioneered ultrasound-guided intratumoral drug delivery with expertise in virology, molecular genetics and immunology, and clinical management of patients with advanced SCLC.

Follow on Funding: None at the time of reporting.

Collaborations: This work includes collaborations between basic scientists and clinicians within the State of Florida and has has previously included collaborations with investigators at the Moffitt Cancer Center who were co-authors on our pre- clinical publication. This work also includes collaborations with the UF Powell Gene Therapy Center and Animal Toxicology Core.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

James and Esther King Biomedical Research Program
Appendix P
Fiscal Year 2020-2021 Active Grants
Funding Year 2016-2017

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
7JK02	H. Lee Moffitt Cancer Center	Christine H. Chung, MD	$1,896,200	2/28/2022	No	Yes	Yes
7JK04	H. Lee Moffitt Cancer Center	Jhanelle E. Gray, MD	$1,895,355	2/28/2022	No	No	No

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

   Principal Investigator: Christine H. Chung, MD

   Organization: H. Lee Moffitt Cancer Center

   Abstract: Head and neck squamous cell carcinoma (HNSCC) remains one of the most devastating cancers in the United States. The sites affected by HNSCC (oral cavity, oropharynx, hypopharynx, and larynx) are critical to the complex and vital functions of speech and swallowing. Therefore, it is clinically challenging because the treatment frequently alters or destroys patients' ability to ailment orally and to communicate verbally. The common risk factors are tobacco and alcohol use and human papillomavirus (HPV) infection. The HPV-positive patients have a favorable outcome given current standard of care compared with patients with HPV-negative, tobacco-related HNSCC. However, even in HPV-positive HNSCC, patients with a history of >10 pack-year of tobacco use have worse outcomes compared to HPV-positive patients without smoking history indicating the devastating effects of tobacco use in treatment resistance and cancer-related death. There are multiple reasons to believe that immunotherapy approaches could be effective in these patients with tobacco-related HNSCC. Current hypothesis is that numerous genetic damage and subsequent mutant proteins in the cancer cells caused by tobacco can be more easily detected by the immune system. Novel immunotherapeutic agents such as the programmed cell-death-1 (PD-1) inhibitors have emerged as a promising therapeutic option. However, only 13-18% of HNSCC respond to these agents, and long-term toxicities have not been fully defined. It is imperative to understand which patients will actually benefit from these agents, to identify ways to improve the current immunotherapy response, and to accurately capture the toxicities as the industry moves towards more personalized therapies.

   In this application, the research team propose: to define the immune microenvironment and neoantigens created by tobacco-related genomic damages in the tumor cells, to generate a molecular signature of the PD-1 inhibitor response, to determine the resistant mechanism and evaluate a combination therapy strategy through a clinical trial, and to develop smart-phone-based assessment of patient-reported outcomes related to receipt of immunotherapy in patients with tobacco-related HNSCC and HPV-positive HNSCC with tobacco exposure. Enrollment of 45 patients with incurable HNSCC who failed prior therapies for the proposed phase I/II clinical

trial of cetuximab and nivolumab in Cohort A has been completed, and the results are published. The enrollment of 43 patients with incurable HNSCC who did not have prior therapy for their recurrent and/or metastatic HNSCC in Cohort B was also completed.

It was found that cetuximab given in combination with nivolumab improves the survival of patients with incurable HNSCC. The response rate was higher in patients with tobacco-related HNSCC compared to human papillomavirus (HPV)-related HNSCC. This is the first therapeutic study to show the combination of cetuximab and nivolumab provides greater benefits in specifically tobacco-related HNSCC. This study results will directly impact how we treat Floridians with incurable, tobacco-related HNSCC. The preliminary data were presented at the American Society of Clinical Oncology Annual Meeting in 2021. A manuscript is being prepared to publish the final results.

Follow on Funding: National Institutes of Health, Xuefeng Wang, $1,249,235.

Collaborations: None at the time of reporting.

Journals: Chaudhary R, Slebos RJC, Song F, McCleary-Sharpe KP, Masannat J, Tan AC, Wang X, Amaladas N, Wu W, Hall GE, Conejo-Garcia JR, Hernanez-Prera JC, Chung CH. Effects of checkpoint kinase 1 inhibition by prexasertib on the tumor immune microenvironment of head and neck squamous cell carcinoma. Mol Carcinog. (2021).

Chung CH, Bonomi M, Steuer CE, Li J, Bhateja P, Johnson M, Masannat J, Song F, Hernandez-Prera JC, Wenig BM, Molina H, Farinhas JM, McMullen CP, Wadsworth JT, Patel KB, Kish JA, Muzaffar J, Kirtane K, Rocco JW, Schell MJ, Saba NF. Concurrent cetuximab and nivolumab as a second-line and beyond treatment of patients with recurrent and/or metastatic head and neck squamous cell carcinoma: Results of phase I/II study. Cancers (Basel). (2021).

Xie M, Lee K, Lockhart JH, Cukras SD, Carvajal R, Beg AA, Flores ER, Teng M, Chung CH, Tan AC. TIMEx: tumor-immune microenvironment deconvolution web-portal for bulk transcriptomics using pan-cancer scRNA-seq signatures. Bioinformatics. (2021).

Patents: None at the time of reporting.

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

Principal Investigator: Jhanelle E. Gray, MD

Organization: H. Lee Moffitt Cancer Center

Abstract: The field of lung cancer is rapidly evolving, however the standard for treating Non-Small Cell Lung Cancer (NSCLC) based on previous trials (KEYNOTE 21G, KEYNOTE 407, and KEYNOTE 189) is triplet combination therapy with platinum doublet plus pembrolizumab. This trial challenges the current landscape by removing chemotherapy and utilizing a novel triplet immunotherapy approach. From July 2020 to June 2021, the clinical study has seen substantial progress in concurrence with its aims, goals, and objectives.

The first aim of this study was to establish the safety and efficacy nivolumab plus/minus nintedanib in both immunotherapy-naïve and pre-treated patients with advanced NSCLC. The Phase I trial successfully identified a recommended safe dose of orally-administered 150 mg Nintedanib daily. Establishment of recommended safe dose allowed study progression into Phase II dose expansion which commenced on May 29, 2019.

The second aim of this study is to confirm whether concurrent administration of nivolumab, ipilimumab, and nintedanib will be efficacious in NSCLC patients in two single arm cohorts: Arm A: newly diagnosed or treatment-naïve patients and; Arm B: patients who have been previously exposed to immunotherapy. Despite COVID-19, diligent efforts have been made to screen, consent, and enroll patients into the dose expansion phase. During this reporting period, phase II has a cumulative total of 56 referrals, 27 patients consented, and 23 patients enrolled (Arm A: 15; Arm B: 8).

The final aim of this study is to examine potential predictive and resistance mechanisms in the tumors of clinical non-responders which is completed through pathology and immune-phenotyping. Pathology has continuously established logistics and quality control of appropriate specimen collection from enrolled patients, established communications with Moffitt's tissue core, CLIA microscopy core, and the mathematical modeling group to plan for biomarker analysis of collected tissue. Pathology is also synthesizing plans for biomarker testing of specimens collected by trial to evaluate for tumor infiltrating lymphocytes, PD-L1, and genetic markers, such as fibroblast growth factor receptor (FGFR) mutations, for correlation with clinical response and resistance. Appropriate specimen collection and processing was implemented for flow cytometric analysis of peripheral blood immune phenotype which continued despite COVID-19. All Phase I patient cohort samples (Patient 001, 002, 003, 006, 007, 009, 010, 011, 012, 013, 014, 015, 017, 018) have been analyzed for their immune phenotype including the maturation status of T cells and expression of immune checkpoints and co-stimulatory molecules. Once treatment response is established, the team will compare responder vs. non-responder.

As NSCLC responses rates vary among patients and tumor types, tumor survival depends, in part, on immune evasion achieve by modifying immune checkpoints. This therapeutic strategy provides potentially meaningful outcomes as a combination therapy that seeks to combat tumor immune resistance thus potentially improving response to NSCLC. As approximately 1.6 million people are diagnosed with lung cancer, of which 85% of cases are NSCLC, the benefit of this combination could provide future opportunities in both treatment-naïve and previously treated patients offering an inclusive option for treatment across the NSCLC spectrum.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

James and Esther King Biomedical Research Program
Appendix Q
Fiscal Year 2020-2021 Completed Grants
Funding Year 2019-2020

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
20K03	Scripps Florida	Thomas Kodadek, PhD	$250,680	5/31/2023	No	No	No

1. **Grant #:** 20K03 Selective Proteolysis of p53 Mis-Sense Mutants

Principal Investigator: Thomas Kodadek, PhD

Organization: Scripps Florida

Abstract: The transcription factor p53 plays a critical role in the development of cancer. p53 is a transcription factor that halts the progression of the cell cycle under conditions of genotoxic stress to allow repair of carcinogenic DNA damage. If the damage is too severe, p53 activates an apoptosis program. Missense mutations in p53 that inactivate the protein are found in more than 50% of all cancers and an even higher percentage of patients with aggressive carcinomas. It is the most commonly found mutation in tobacco-related cancers, especially in the lung.

By far the most common cancer-driving point mutations in p53 are located within the DNA-binding domain (DBD). These mutations destabilize the domain thermally. It is misfolded at 37oC, and thus is unable to bind to DNA and unable to activate transcription. This would be bad enough, but the more serious problem is that the mis-sense p53 mutants accumulate to very high levels in cancer cells and display both dominant negative behavior and toxic gain of functions that are strongly tumor promoting. A drug capable of blocking these functions of mutant p53 proteins would be transformative for cancer chemotherapy.

Our research team proposes to develop a (proteolysis targeting chimera) PROTAC able to engage p53 mis-sense mutants. PROTACs are chemical dimerizers capable of forcing a target protein and an E3 ubiquitin ligase (Ubl) complex into close proximity, resulting in poly-ubiquitylation and subsequent proteasome-mediated degradation of the target. PROTACs targeting many different proteins have been reported over the last few years, most of which contain existing ligands for the VHL or Cereblon E3 ubiquitin ligases. Therefore, the seminal issue in developing a p53 mis-sense mutant-targeted PROTAC is identifying a ligand for the mis-folded DBD of the mutant protein. Moreover, it is critical to find a ligand that cleanly distinguishes the mutant protein from wild-type p53, since knocking down the level of functional p53 is obviously not a good idea. The team will screen novel DNA-encoded libraries developed recently in this laboratory to identify the appropriate ligand. These molecules will then be conjugated to an E3 ubiquitin ligase ligand to create a PROTAC that will be tested in various cancer cell lines.

The major goals of this project have not been met due to the early termination of funding. Our near-term goal, was to conduct a high-throughput screen of a DNA-encoded library for ligands that would bind to p53 missense mutants, but not to wild-type p53. The team produced recombinant p53 DNA-binding and tetramerization domain constructs in mammalian cells (to assure proper post-translational modification) and demonstrated that this construct binds with high affinity to a fluorescently labeled DNA oligonucleotide containing a p53 binding site. This complex was to be the target of the screen, which would also include misfolded p53 (not bound to DNA) as an off-target. A high-quality DNA-encoded library was prepared containing approximately 760,000 unique macrocycles. Unfortunately, just as the team was poised to conduct the screen, funding was terminated.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
8JK01	Florida Atlantic University	Gregg B. Fields, PhD	$708,044	4/30/2021	No	Yes	No
8JK02	H. Lee Moffitt Cancer Center	Jennifer B. Permuth, PhD	$1,360,857	3/31/2021	No	Yes	No
8JK05	University of Florida	Sergei Kusmartsev, PhD	$816,514	3/31/2021	No	Yes	Yes
8JK06	University of Florida	Sergei G. Tevosian, PhD	$816,514	3/31/20201	No	No	No
8JK07	University of Miami	Sabita Roy, PhD	$816,514	3/31/2021	No	No	No
8JK09	University of South Florida	Tomar Ghansah, PhD	$816,514	03/31/201	Yes	Yes	No

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

   Principal Investigator: Gregg B. Fields, PhD

   Organization: Florida Atlantic University

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

of the effects of binding partners on MT1-MMP activity, and Screening of improved inhibitors in cell-based and spheroid invasion assays.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Characterization and regulation of MT1- MMP cell surface- associated activity Sonia Pahwa, Sabrina Amar, Manishabrata Bhowmick, Jian Cao, Alex Y. Strongin, Rafael Fridman, Stephen J. Weiss, and Gregg B. Fields. Chemical Biology & Drug Design. (2019). Vol. 93, pages 1251- 1264.

A novel probe for spliceosomal proteins that induces autophagy and death of melanoma cells reveals new targets for melanoma drug discovery. Manikandan Palrasu, Anna M. Knapinska, Juan Diez, Lyndsay Smith, Travis LaVoi, Marc Giulianotti, Richard A. Houghten, Gregg B. Fields, and Dmitriy Minond Cellular Physiology and Biochemistry. (2019) Vol. 53, pages 656-686.

Patents: None at the time of reporting.

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

Principal Investigator: Jennifer B. Permuth, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: Of all tobacco-related cancers in the United States, pancreatic cancer (PC) is the deadliest, with a five-year relative survival rate of only 9%. PC just became the third leading cause of cancer deaths and will become the second leading cause around 2020. Florida ranks second in lives lost to PC each year. Striking racial disparities in PC incidence and mortality rates exist nationally and in Florida, with the highest rates among African Americans (AA) followed by Non-Hispanic Whites (NHW) and Hispanic/Latinos (H/L). Reasons for these disparities remain unexplained and underexplored. One factor that contributes to increased morbidity and mortality and diminished quality of life (QoL) in most PC patients is cancer cachexia, a metabolic condition characterized by stages of progressive muscle wasting, unintentional weight loss, and fatigue. The goal of this infrastructure grant is to create state resources to conduct basic, clinical, population-based, and translational science that will impact several racial and ethnic groups affected by PC. PC researchers from fifteen Florida cancer centers and hospitals that diagnose and treat a high volume of AA, NHW, and/or H/L individuals with PC have joined forces to: prospectively build a robust ‘next-generation biobank’ that contains viable tissues, biofluids, medical images, and clinical and laboratory data, all derived from a racial/ethnically diverse cohort of PC patients, and use the biobank to test the hypothesis that cancer cachexia may underlie racial disparities in PC such that AA may present with a higher prevalence of cachexia earlier and more aggressively in the disease process compared to NHW and H/L. The research team has been productive in building the foundation for this

infrastructure project as evidenced by accomplishments in numerous areas including: meeting with scientific and community advisors and co-investigators to discuss and enhance the scope of work; finalizing the study protocol, master consent form, study questionnaires and numerous data collection instruments, and translating pertinent documents into Spanish; obtaining regulatory approval and executing various contracts and agreements; and harmonizing standard operating procedures related to biospecimen and medical image collection, processing, storage, and transfer. The team also built a customized platform for data collection, management, and workflow and developed a study logo, recruitment materials, and a study web-site. To date, more than 450 participants have been recruited, and have donated data, images, and biospecimens for this state-wide biorepository. This infrastructure has the potential for great impact because it will address a critical gap in PC research by capitalizing on Florida's large underserved minority PC population and an already established and productive multidisciplinary collaboration with new passionate partners.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Permuth JB, Dezsi KB, Vyas S, Ali KN, Basinski TL, Utuama OA, Denbo JW, Klapman J, Dam A, Carballido E, Kim DW, Pimiento JM, Powers BD, Otto AK, Choi JW, Chen DT, Teer JK, Beato F. Ward A, Cortizas EM, Whisner SY, Williams IE, Riner AN, Tardif K, Velanovich V, Karachristos A, Douglas WG, Legaspi A, Allan BJ, Meredith K, Molina-Vega MA, Bao P, St. Julien J, Huguet KL, Green BL, Odedina FT, Kuman NB, Simmons VN, George TJ, Vadaparampil ST, Hodul P, Arnoletti JP, Awad ZT, Bose D, Jiang K, Centeno BA, Gwede CK, Malafa M, Judge SM, Judge AR Jeong D, Bloomston M, Merchant NB, Fleming JB, Trevino JG. The Florida Pancreas Collaborative Next-Generation Biobank: Infrastructure to Reduce Disparities and Improve Survival for a Diverse Cohort of Patients with Pancreatic Cancer. Cancers (Basel). (2021). doi: 10.3390/cancers13040809. PMID: 33671939 PMCID: PMC7919015

Patents: None at the time of reporting.

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

Principal Investigator: Sergei Kusmartsev, PhD

Organization: University of Florida

Abstract: The overall goal of current proposal is to examine the specific mechanisms underlying hyaluronan-mediated formation of immunosuppressive Programmed Death Ligand 1 (PD-L1)-expressing macrophages, regulation of PD-L1 expression and to investigate the therapeutic potential of targeting hyaluronan-CD44 link for the treatment of bladder cancer using an experimental tumor model and cancer patients. Two specific aims are designed to achieve a proposed goal. First, the mechanistic part of the study will be focused on the molecular mechanisms by which tumor-derived hyaluronan promotes formation of immunosuppressive

PD-L1-expressing macrophages. In the second part of this study, the research team plans to evaluate the therapeutic anti-tumor activity of targeting hyaluronan synthesis and CD44 receptor in bladder cancer. Inhibiting of hyaluronan synthesis by tumors or targeting hyaluronan-mediated CD44 signaling could provide an attractive approach to break tumor-induced immune tolerance and unleash the anti-tumor immune response. Data obtained in the course of this study, allowed us to make significant progress regarding the understanding roles of hyaluronan in tumor development and progression. It is important to note that this study has led to the discovery of the novel enzymatic function of tumor-associated Hyal2+ myeloid cells which greatly contributes to enhanced degradation of tumor- associated hyaluronan and accumulation of highly fragmented low molecular weight hyaluronan in tumor tissue. Small Hyaluronan (HA) fragments strongly stimulate cancer-related inflammation and tumor angiogenesis through Toll-like Receptors (TLR)-dependent signaling. After completing the current project, the team plans to prepare and submit new grant proposals to federal agencies such as National Institute of Health/National Cancer Institute and Department of Defense.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Mechanisms of Immune Evasion in Bladder Cancer Crispen P. and Kusmartsev S. Cancer Immunology and Immunotherapy.(2020).

Hyal2 Expression in Tumor- associated Myeloid Cells Mediates Cancer-related Inflammation in Bladder Cancer. Dominguez Gutierrez P.R, Kwenda E, Donelan W, O' Malley P, Crispen P, and Kusmartsev S. Cancer Research. (2020).

Detection of PD-L1- expressing myeloid cell clusters in the hyaluronan- enriched stroma in tumor tissue and tumor-draining lymph nodes. Dominguez Gutierrez P.R, Kwenda E, Donelan W, O' Malley P, Crispen P, and Kusmartsev S. Journal of Immunology. (2021).

Patents: None at the time of reporting.

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

Principal Investigator: Sergei G. Tevosian, PhD

Organization: University of Florida

Abstract: Based on the results obtained with the offspring, the research team thought to expand analysis of gene expression in the maternal cecal tissue, to strengthen the connection between gene expression, pregnancy and nicotine-induced changes. The aim was to identify additional genes that are specifically affected by pregnancy, nicotine exposure or both.

Relative gene expression in the female cecal tissue that were primarily altered in response to pregnancy only included an up-regulation of the tight junction regulator occludin ($P<0.04$; two-way ANOVA), down regulation of the sodium-coupled monocarboxylate transporter 1 (smct1 or

SLC5A8; P<0.02) linked to short-chain fatty acid (SCFA) transport, and a strong trend for up-regulation of mucin 3 (muc3; P<0.06) linked to gut epithelial lining protection. The expression of those genes that were primarily altered by NIC; interestingly, in all three instances NIC induced a down-regulation of gene expression, including that of GCG or preproglucagon (P<0.019; two-way ANOVA), GLP-1, the receptor for glucagon which has been linked to regulating both gut motility and inflammation (P<0.003), and spontaneously immrtalized monocyte-like cell line (TPH1), the rate limiting enzyme in the production of serotonin by enterochromaffin cells in the gut and linked to maintenance of gut health (P<0.015). The three genes altered by both pregnancy and NIC exposure. Included in this group was 11-beta-Hsd2, which is involved in metabolizing glucocorticoids and was up-regulated by pregnancy (P<0.043; two-way ANOVA) but was down-regulated by NIC (P<0.05).

The exact role of 17 beta-hydroxysteroid dehydrogenase type 2 (HSD2) in the gut is not understood, but may be linked to increased metabolism of cortisol, potentially modulating fetal exposure to stress hormones during pregnancy, similar to its role in the placenta. Ffar2, or free fatty acid receptor 2 (Gpr43) was also up-regulated by pregnancy (P<0.005) but down-regulated by NIC (P<.0018). This pattern is similar to that observed for its primary ligands, the cecal SCFAs acetate and propionate. Additional genes were evaluated but did not show any change in pregnancy and/or NIC; they included Tumor Necrosis Factor (TNF) alpha (P>0.42), lactate transporter (P>0.08), sert1 (P>0.1), and Ffar3 (P>0.38). This new data will be included in manuscript currently in preparation.

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 #:** 8JK07-A1 Evaluating Mechanisms of Stromal Modulation by Novel Anti-Cancer Drug Minnelide

Principal Investigator: Sabita Roy, PhD

Organization: University of Miami

Abstract: Pancreatic cancer is an aggressive malignancy characterized by a dense stroma which makes it recalcitrant to standard chemotherapeutic or surgical treatment strategies. Smoking is considered to be one of the factors that contributes to a complex tumor microenvironment in making this disease aggressive and difficult to treat. Minnelide, a water-soluble pro-drug of triptolide, developed by this group, has recently completed Phase I clinical trials and is currently awaiting Phase 2 trial. Preliminary data suggests that at a low dose 0.2 mg, Minnelide depletes the stromal extracellular matrix components (ECM), thus relieving the interstitial pressure on the blood vessels and leading to better drug delivery. Based on this observation, the team hypothesizes that at a lower dose, Minnelide inactivates the cancer

associated fibroblasts (CAFs), pushes them to quiescence and modulates the tumor-stroma-immune crosstalk. Furthermore, the team hypothesizes that a combination of this stromal modulator effects of Minnelide combined with standard- of care drugs such as gemcitabine should lead to strong anti-tumor effects. This hypothesis was tested by the following three aims: Evaluation of the effect of combination of Minnelide and gemcitabine in animal models. Elucidation of the mechanism by which Minnelide induces Stromal reprogramming. Determine the effect of stromal modulation by Minnelide on oncogenic pathways in tumor cells and pro-tumorigenic immune pathways. Overall, the studies show that Minnelide reprograms some of the important components of stroma and ECM which could account for its anti-tumor effects. Results also show that Minnelide in combination with standard-of-care-drugs is also quite effective in reducing the tumor size in our syngenic mouse models. In this context, the innovation of the proposal lies in the following aspects: a unique combination of two compounds; one that is known to target tumor epithelial cells, gemcitabine and Minnelide that mediates stromal re-programming in the animal models to develop a viable therapeutic option.

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

Principal Investigator: Tomar Ghansah, PhD

Organization: University of South Florida

Abstract: From April 2018 to December 2020, respectively, this research team generated all of the data. In fact, the team was able to garner the necessary data (i.e. pancreatic cancer [PC] orthotopic mice were treated with and without application programming interface (API) and then monitored with Vevo Ultrasound Image system) in order to complete a PLoS One manuscript that was submitted in January 2019. This manuscript was not accepted because the reviewers requested more data and the team needed to generate more heterotopic PC Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1 (SHIP) knockout (KO) and SHIP wild type (WT) mice to improve to reproducibility of the results. The manuscript was revised and submitted it to the Journal of OncoImmunology in June 2019 and it was not accepted. Therefore, based on the comments of OncoImmunology reviewers, the team revised the manuscript and submitted to Cancers in August 2020. We received a notification that manuscript will be accepted pending revisions in October 2020. On December 4, 2020, the manuscript was published entitled “Apigenin Increases SHIP-1 Expression, Promotes Tumoricidal Macrophages and Anti-Tumor Immune Responses in Murine Pancreatic Cancer”.

The world-wide pandemic, due to COVID-19 (which started mid-March 2020) research productivity in the lab has slowed. Fortunately, the team acquired a majority of the data need for this manuscript to be completed with the exception of a few key experiments. The publication of research in cancers is one of our most significant scientific accomplishments to date. Before the submission of Cancers manuscript for publication, the team was able to submit an NIH NCI R01 application in June 5, 2020. This grant was reviewed but not scored. However, after speaking with the Program Officer, she stated based on the reviewer's comments, she encouraged the team to resubmit once the peer-reviewed publication was accepted and focus on Aims 1-2 but remove Aim 3 for the R01 grant application. There are plans to resubmit this revised NIH NCI R01 application.

Dr. Husain has made a major contribution regarding the SHIP transgenic mice that helped this research (Team members Krystal Villalobos-Ayala (former USF Master Student and now outstanding and experienced Senior Tech), Bradley Miller (USF undergraduate student), Adriana Morales Rivera (USF master internship student) and Chukwuebuka Eburuoh (rotation Ph.D. student) and together sped up momentum to address Aims 1-2 in this study. In addition, Dr. Husain has more than 15 years of working with pancreatic cancer and generated different transgenic tumor-bearing models of pancreatic cancer and molecular biology techniques are highly appreciated. Therefore, he is an essential member of the team and has helped garner the data needed for Aims 1-2 and the recent publication in Cancers. Dr. Husain has helped with the completion of review article "Protein kinase 2 (CK2): a potential regulator of immune cell development and function in cancer" that was accepted in November 2020 to the Journal of Immunological Medicine. Additionally, of more manuscripts and external grants (i.e. NIH NCI) are anticipated in 2021.

Follow on Funding: None at the time of reporting.

Collaborations: Jason B. Fleming, M.D. from Moffitt Cancer Center (Department Chair of GI Oncology and Tampa, FL) and Jose Trevino, M.D. from the University of Florida (College of Medicine/Department of Surgery and Gainesville, FL) for the purpose of gaining access to patient-derived xenograft (PDX) mice. PDX mice will prove to be beneficial for our pre-clinical studies by providing more realistic outcomes for potential immunotherapy treatments.

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

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

Gerald Krystal, Ph.D. (College of Medicine/Department of Pathology & Laboratory Medicine, and Vancouver, BC) and Laura Sly, Ph.D. (College of Medicine/Department of Pediatrics and Vancouver, BC) from the University of British Columbia provided transgenic and wild type mice for on-going experiments.

Krystal Villalobos-Ayala, M.S., is a full-time Lead Research Technician in my lab. Krystal has been working on in vitro assays (i.e. T cell activation assays and Allogenic Mixed Lymphocyte Reactions) using our SHIP mice and OPC models.

Journals: Villalobos-Ayala, K., Alvarez, C., Ortiz Rivera, I., DeLoach, D., Miller, B., Husain, K., Krystal, G., Hibbs, M., Jiang K., Ghansah T. Apigenin Increases SHIP-1 Expression, Promotes Tumoricidal Macrophages and Anti-Tumor Immune Responses in Murine Pancreatic Cancer. (2020).

Husain K, Williamson TT, Nelson N, Ghansah T. Protein kinase 2 (CK2): a potential regulator of immune cell development and function in cancer. Published to Immunological Medicine.(2020).

Husain, K., Villalobos-Ayala K., Ghansah T. Apigenin Targets microRNA-155, Enhances SHIP-1 Expression, Improves Myelopoiesis and Anti-tumor Responses in Pancreatic Cancer. In preparation to OncoImmunology.

Villalobos-Ayala, K., Luongo, J., Marsh, A., Areas, J., Miller, B., Husain, K., Ghansah, T. Apigenin modulates Immune Checkpoint Molecules in Pancreatic Cancer enhancing Anti-tumor Immunity. In preparation to OncoImmunology.

Patents: Apigenin Targets Microrna-155, Enhances Ship-1 Expression, Improves Myelopoiesis And Anti-Tumor Responses In Pancreatic Cancer, 63/202,799 University of South Florida

Apigenin Increases SHIP-1 Expression Impact Anti-tumor Immune Responses in Pancreatic Cancer ID#: 20B203, University of South Florida

James and Esther King Biomedical Research Program
Appendix S
Fiscal Year 2020-2021 Completed Grants
Funding Year 2016-2017

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
7JK01	University of Miami	Helen M. Bramlett, PhD	$1,253,753	2/28/2021	No	Yes	Yes
7JK03	University of Miami	W. Dalton Dietrich, PhD	$1,895,355	2/28/2022	No	Yes	No
7JK05	University of Florida	Zhihua Jiang, PhD	$1,422,150	2/28/2021	No	Yes	Yes

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

   Principal Investigator: Helen M. Bramlett, PhD

   Organization: University of Miami

   Abstract: Millions of smokers are disabled as a result of stroke in the United States. Stroke disproportionately kills more women than men. It is now known that smoking is a predictor of frailty and pre-stroke smoking is associated with increased post-stroke frailty. Although frailty is associated with increased in-hospital mortality, poorer outcome at discharge, the prevention and treatment of smoking and stroke associated frailty remains unaddressed. This proposal targets the prevention and treatment of post-stroke frailty by studying the impact of smoking cessation and an intervention on stroke outcomes. A growing body of evidence in animals and humans has shown that whole body vibration (WBV) reduces or reverses pathology and such treatment also may help improve frailty-related stroke physiological deterioration. The goal of this current study is to investigate the effects of WBV on ischemic outcomes in female rats exposed to smoking attributed nicotine. The research team proposes the following aims to test the hypotheses.

   Aim 1: To determine the effect of WBV on the neurobehavior of nicotine exposed rats after stroke. Staff hypothesize that WBV will reduce frailty and improve cognition in nicotine exposed female rats after tMCAO.

   Aim 2: To determine the effect of WBV after stroke on circulatory endothelial progenitor cells (EPCs) and inflammatory markers in the blood of nicotine exposed female rats. Staff hypothesize that WBV will increase circulating EPCs and reduce inflammatory cytokines and inflammasome activation after stroke.

   Aim 3: To determine the effect of WBV on neurogenesis in the brain of nicotine exposed female rats after stroke. Staff hypothesize that WBV after stroke will increase the release of growth factors and stimulate endogenous neurogenesis. These proposed studies will identify mechanisms by which WBV reduces frailty and improves stroke outcomes, and provide a basis for future targeted interventions to enhance recovery after stroke in women smokers. Progress

from this grant has provided sufficient preliminary findings to submit for a Florida Biomedical Grant assessing the use of therapeutic hypothermia in combination with whole body vibration after nicotine exposed rats stroke rats.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Sexually dimorphic microglia and ischemic stroke. Kerr N, Dietrich WD, Bramlett HM, Raval AP. CNS Neuroscience & Therapeutics. (2019).

Simultaneous nicotine and oral contraceptive exposure alters brain energy metabolism and exacerbates ischemic stroke injury in female rats. Diaz F and Raval AP. J Cereb Blood Flow Metab.(2020) 14:271678X20925164.

Nicotine exposure along with oral contraceptive treatment in female rats exacerbates post-cerebral ischemic hypoperfusion potentially via altered histamine metabolism. d'Adesky N, Diaz F, Zhao WZ, Bramlett HM, Perez-Pinzon MA, Dave KR, and Raval AP. Translational Stroke Research. (2020).

Osteocalcin, ovarian senescence, and brain health. Schatz M, Saravanan S, d'Adesky ND, Bramlett H, Perez-Pinzon MA, Raval AP. Front Neuroendocrinol. (2020).

Patents: None at the time of reporting.

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

Principal Investigator: W. Dalton Dietrich, PhD

Organization: University of Miami

Abstract: This research project's progress of successfully demonstrating that pathophysiological mechanisms may be sex-dependent and thereby influence the effectiveness of specific therapeutic treatments indicates that there are gender differences between infarct size between non-treated male and female rats. However, female animals treated with P7C3-A20 displayed similar infarct sizes compared to non- treated female rats. Interestingly, vehicle treated females with transient middle cerebral artery occlusion (tMCAO) had the smallest cortical volume compared to all other groups. Aims 1 and 2 have been completed. For Aim 3 as previously reported, the team has moved to a cortical photothrombotic model of acute stroke for the mouse studies described in Aim 3. Initial findings of hippocampal neurogenesis were produced using the transgenic nestin green fluorescent protein (GFP) mice with this model and demonstrating behavioral deficits. However, there was one set back with the cytovene pumps to ablate the nestin positive neurons as previously reported in a quarterly report. Additionally, the team encountered another setback when the current laser failed and a new laser needed to be purchased. The team is continuing to produce pilot animals to verify the cortical infarct. Due to COVID-19, the team had to discontinue this work, but have since started back re-verifying the infarcts. The team was able to produce cortical infarcts with our new model. However, due to

COVID-19 and laser issues, the team was unable to produce animals using Cytarabine (ARA-C) to knockdown neurogenesis. Some key personnel left the university, but they have been replaced, and there have been no delays due to personnel changes. There are plans to submitt a grant investigating the impact of Alzheimer's disease (AD) in our new photothrombotic stroke model. The results that were produced from this grant will provide preliminary data producing a stroke in AD transgenic mice.

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

Principal Investigator: Zhihua Jiang, PhD

Organization: University of Florida

Abstract: This project was initially funded as a three-year project. It was extended at no cost for an additional year due to the slowdown of project progress caused by the COVID-19 pandemic. The project staff are very grateful for such a professional administrative management, which provided sufficient time to complete the proposed studies. Completion of these studies has led to several major accomplishments, including novel scientific findings, growth of the infrastructure, and success in obtaining research funds from the National Institutes of Health. The preliminary data obtained from this project suggests that tobacco smoke primes smooth muscle cells (SMCs) for an inflammatory phenotype by disrupting actin dynamics, rendering aortas vulnerable to structural degeneration and aneurysm formation. Abdominal aortic aneurysms (AAAs) are present in 5% of the population aged > 65 years. The incidence of AAAs is three-five times greater in smokers than in non-smokers. In addition, tobacco smoke (TS) doubles the rate of aortic expansion and the risk of aortic rupture. Despite this well-established importance, little is known about the mechanisms by which TS exacerbates AAA formation. As a result, medical interventions to reduce the risk of AAAs for tobacco users remain unavailable. Recent advances in the pathophysiology of AAA formation have led to a revised concept that AAA is a local manifestation of a systemic disease process. Specifically, evidence obtained from epidemiological investigations and experimental studies suggest a modulatory role for the imbalanced T-helper 1/ T-helper 2 (TH1/TH2) immunity in AAA development, with the TH2 dominated immune response being destructive to the aortic wall. Intriguingly, emerging evidence demonstrates that TS skews the TH1/TH2 balance to a TH2 immunity. These lines of evidence have led the team hypothesize that a skewing of the TH1/TH2 balance to TH2 immunity underlies TS-exacerbation of aortic aneurysms. The group has recently developed a mouse model where chronic nicotine infusion increases the incidence of AAAs from 6% to 60%. With this powerful tool, the current project seeks to: Understand the dynamic drifting of the TH1/TH2 balance in aneurysmal aortas exposed to chronic nicotine infusion; Determine the TH2

polarization as a mechanism for ten of twelve nicotine-exacerbation of AAA formation using genetic approaches; and Evaluate the effect of pharmaceutical inhibition of TH2 polarization on AAA development. Results obtained from this project will: provide detailed information on the modulation of T-cell differentiation, macrophage polarization, and cytokine profile by nicotine over the course of AAA development; and offer mechanistic understanding of the role of major T-cell-transcription factors and signature cytokines in mediating nicotine-exacerbation of AAA formation. An R01 proposal will be prepared for submission once the aforementioned two papers are published.

Follow on Funding: None at the time of reporting.

Collaborations: Dr. Gilbert R. Upchurch Jr. is an internationally known expert in the filed of research for aortic aneurysms. He is also the Chair of the department. We have been having joined lab meetings weekly and meeting in person monthly. We also work together to publish papers and write grants.

Andrew J. Bryant. Dr. Bryant is a physician of the Division of Pulmonary, Critical Care and Sleep Medicine. He is expert in the field of pulmonary hypertension.

Laurence M. Morel. Dr. Morel is an Immunologist and Mary and Bryan Whisenant Professor of Pathology.

Yong Huang. Dr. Huang is professor in the Department of Engineering. We collaborate on a project focusing on engineering of vascular grafts.

Journals: Cyclophilin A contributes to aortopathy induced by postnatal loss of smooth muscle TGFBR1. Zhou G, Liao M, Wang F, Qi X, Yang P, Berceli SA, Sharma AK, Upchurch GR Jr, Jiang Z. FASEB J. (2019).

A validated mouse model capable of recapitulating the protective effects of female sex hormones on ascending aortic aneurysms and dissections (AADs). Xiaoyan Qi, Fen Wang, Changzoon Chun, Lennon Saldarriaga, Zhisheng Jiang, Eric Y. Pruitt, George J. Arnaoutakis, Gilbert R. Upchurch, Jr, Zhihua Jiang. Physiol Rep (2020).

Nicotine exacerbates TAAD formation under optimized experimental conditions. Changzoon Chun1, Xiaoyan Qi1, Fen Wang1, Kyle B. Marid1, Lennon A. Saldarriaga1, Max R. Fisch1, Mark L. Brantly2, Gilbert R. Upchurch Jr1, and Zhihua Jiang1 American Journal Physiology-Heart and Circulatory Physiology.

Patents: None at the time of reporting.

James and Esther King Biomedical Research Program
Appendix T
Fiscal Year 2020-2021 Completed Grants
Funding Year 2015-2016

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
6JK02	H. Lee Moffitt Cancer Center	Vani N. Simmons, PhD	$1,186,164	2/28/2021	No	Yes	No
6JK04	Florida International University	Maria Jose Miguez, MD, PhD	$1,628,449	2/28/2021	No	Yes	No

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

   Principal Investigator: Vani N. Simmons, PhD

   Organization: H. Lee Moffitt Cancer Center

   Abstract: Cigarette smoking remains the top avoidable cause of death and disease in Florida, responsible for most cases of lung cancer, as well as many other cases of cancer, heart disease, pulmonary disease, and diabetes. Despite the state-supported availability of multiple avenues for smoking cessation (e.g., telephone counseling, web-assisted cessation, in-person counseling, free nicotine replacement therapy), only a small percentage of smokers within Florida avail themselves of these treatment options, and approximately 18% of Floridian adults continue to smoke. Research over the past several decades has shown that long-term cessation rates with even the most intensive interventions rarely exceed 20-30%.

   Thus, it is vital that additional research be conducted to develop and validate novel methods for effective smoking cessation. It has long been understood that nicotine is the primary constituent in cigarettes and other tobacco products that supports the initiation and maintenance of an addiction to tobacco. The recent availability (via the NIDA Drug Supply Program) of research cigarettes with varying levels of nicotine creates a unique opportunity to evaluate the potential benefits of very low nicotine content (VLNC) cigarettes as a new tool for smoking cessation. In that vein, the proposed research will develop and test a novel smoking cessation strategy, based on theory and research concerning extinction. Specifically, this theory-driven intervention will be designed to extinguish the expectation of reinforcement from smoking via pre-quit smoking of VLNC cigarettes. In turn, this should result in a higher likelihood of successful quitting. First, the experienced research team and consultants will adapt and refine intervention materials, in order to provide smokers with clear and detailed instructions for smoking VLNC cigarettes prior to quitting, in a manner that will maximize extinction to smoking-related reinforcement. This intervention development process will involve expert review and recommendations, and will incorporate feedback from smokers (n=20) enrolled in a pilot study of the intervention. The pilot study will also examine the feasibility of the targeted intervention, along with two tapering schedules (immediate vs. gradual) for transitioning to VLNC cigarettes over a four-week pre-quit period. Next, a randomized controlled trial (RCT; n=200) will compare

effects of the targeted intervention vs. a standard intervention, in combination with the two tapering schedules, on smoking cessation outcomes. In addition, two behavioral paradigms will be administered to RCT participants, to determine intervention effects on smoking behavior in a short-term laboratory Smoking Analogue tasks and a five-day abstinence challenge task. Finally, analyses will explore if the treatment works better for particular types of smokers, and if the treatment effects on smoking cessation outcomes are mediated by effects on related variables (e.g., decreased nicotine withdrawal or cravings to smoke). This information will be particularly useful for determining which smokers may benefit most from this smoking cessation approach, and for better understanding how this form of treatment works. The study team has successfully concluded recruitment for the RCT providing data to complete the study aims of this grant. All follow-up visits were also completed within this timeframe, with the final assessment conducted on 08/24/2021. A total of 147 participants were randomized to treatment: 72 participants were randomized to the targeted group and 75 to the control group. Moreover, a total of 75 participants were randomly assigned to the immediate nicotine reduction group and 72 to the gradual group. Findings regarding smoking cessation outcomes are not yet available as function of condition, given the recent completion of data collection.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Facilitating smoking cessation using reduced nicotine cigarettes: Intervention development and RCT study design. Conn, M.R., Brandon, T.H., Lorenzo, Y.L., Sawyer, L.E., Simmons, V.N., Sutton, S.K., Donny, E.C., Hatsukami, D., & Drobes, D.J. Contemporary Clinical Trials. Published. (2020).

Patents: None at the time of reporting.

2. **Grant #:** 6JK04 Biobehavioral Intervention For Smokers Living With HIV

Principal Investigator: Maria Jose Miguez, MD, PhD

Organization: Florida International University

Abstract: Most theories related to health behavior changes (e.g. health belief model, transtheoretical model, social cognitive theory) posit that high levels of knowledge and perceived vulnerability are associated with greater intentions and greater likelihood of quitting smoking. On the other hand, when the smoker inaccurately believes they are at lower risk than their counterparts also known as “optimistic bias” they are less likely to quit. Therefore, one of our aims based on those principles was increase knowledge and risk perception. The next analyses examine the degree to which there were improvements in these areas and if they prospectively related to smoking cessation. Approximately 25% of the deaths in the United States are due to smoking-related morbidity, and the financial burden of smoking exceeds $289 billion each year. As dire as the risk of smoking appears for the general population, the impact of tobacco among those living with HIV/AIDS (PLWHA) is substantially worse. Unfortunately, while nearly two-thirds of HIV+ smokers wish to quit smoking, their rate of success is lower than

the one among the general population. The typical HIV-infected smoker is considered “difficult to treat” because of very high rates of psychiatric and substance use comorbidities [9]. Such patients generally require intensive interventions in order to quit. In average rates of smoking cessation achieved using recommended brief interventions and some pharmacotherapy are ranging between 4-20%. With two caveats, clinical trials were generally small and those rates were attained at three months, very few at six months and even less at 12 months. In contrast this clinical trial is one of the largest studies with long follow-ups. With a response rate of 98%, four hundred eighty-eight HIV infected smokers were enrolled, and randomized. A total of 271 men and 216 female smokers were enrolled until 12/2018. The advancement in the antiretroviral therapy (ART) has drastically reduced morbidity and mortality; however, it raises the burden of developing comorbidities such as cancers. Women living with HIV (WLWH) are affected by gynecological and non-gynecological malignancies at disconcerting rates. Among women living with HIV (WLWH) the risk of developing anal and cervical cancer (AC, CC) is several folds higher than in their seronegative counterparts and their rates of abnormal anal cytology are even higher. As a result, treatment strategies are focusing on promoting their longevity. With this new framework, clinicians and health authorities are focusing on detection of malignancies such as cervical and anal cancer that are associated with certain viral infections, with chronic inflammation, and with lifestyle choices. In 2018, both the United States President’s Emergency Plan and the UNAID launch a program to assure that “all women living with HIV need access to information on HPV and should be offered cervical cancer screening and treatment if necessary.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Maria Jose Miguez, M.D., Ph.D. (Principal Investigator), Diego Bueno., MS, Clery Quiros, MS. Gender differences in smoking behaviors are narrowing but health impact is wider in people living with HIV. Clinical Case Studies & Reviews. (2020).

Miguez MJ, Bueno D and Quiros C. Health Disparities on COVID-19: The Need of a Holistic Model That Must Recognize the Biology Perspective. SunText Review of Virology.

Maria Jose Miguez, Calonie Gray, Jose Castro, Cassandra Stanton, Clery Quiros, Diego Bueno, Christopher Kahler. Mentholated cigarettes or weight problems, which came first. Adv Obesity Weight Management Control. (2018).

Management and Media Studies. Miguez MJ, Bueno D and Quiros C. “What We Do Not Know About Smoking and COVID-19 Can Hunt Us Down”. Cross Current International Journal of Economics.

Patents: None at the time of reporting.

James and Esther King Biomedical Research Program
Appendix U
Fiscal Year 2020-2021 Completed Grants
Funding Year 2014-2015

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
5JK02	University of Miami	Michael Campos, MD	$1,951,531	5/15/2020	No	Yes	Yes
5JK03	H. Lee Moffitt Cancer Center	Vani Nath Simmons, PhD	$1,904,351	5/15/2020	No	Yes	Yes

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

   Principal Investigator: Michael Campos, MD

   Organization: University of Miami

   Abstract: In 2016, this research team published a paper in Thorax [Garcia-Arcos I, Geraghty P, Baumlin N, Campos M, Dabo AJ, Jundi B, Cummins N, Eden E, Grosche A, Salathe M, Foronjy R. Chronic electronic cigarette exposure in mice induces features of COPD in a nicotine-dependent manner. Thorax 2016;71(12):1119-1129]. This paper was accompanied by an editorial discussing the possible health impact of e-cigarettes (Shapiro SD, Kaynar AM. Electronic cigarettes: the lesser of two evils, but how much less? Thorax 2016;71:1080-1081). The article has been cited 187 times. The paper was a collaboration between this in vitro work (funded by J&E King) and Dr. Foronjy's in vivo work. The team showed in vitro that e-cigarette vapor reduced parameters of mucociliary function. In vivo, vapor caused airway inflammation and emphysema. Importantly, the vaping mechanism was analyzed to see whether one can predict those that can successfully quit tobacco use with e-cigarettes. In 2018, the team published a groundbreaking paper on vaping topography (Guerrero-Cignarella AM, Diaz LVL, Balestrini K, Holt G, Mirsaeidi M, Calderon-Candelario R, Whitney P, Salathe M, Campos MA. Differences in vaping topography in relation to adherence to exclusive electronic cigarette use in veterans. PLoS ONE 2018;13(4):e0195896). Interestingly, it seems that people who quit successfully take longer vapor puffs through e-cigarettes than the ones who failed. The article has been cited eight times. In 2019, the team published a paper describing the effects of vaping on mucociliary function in the American Journal of Respiratory and Critical Care Medicine, the premier journal for clinical and translational pulmonary research (Chung S, Baumlin N, Dennis JS, Moore R, Salathe SF, Whitney PL, Sabater J, Abraham WM, Kim MD, Salathe M. Electronic cigarette vapor with nicotine causes airway mucociliary dysfunction preferentially via TRPA1 receptors. American Journal of Respiratory and Critical Care Medicine 2019;200(9):1134-1145). This paper was accompanied by an editorial discussing the impact of nicotine-containing e-cigarette vapor on mucociliary clearance (Kesimer M. Another warning sign: high nicotine content in electronic cigarettes disrupts mucociliary clearance, the essential defense mechanism of the lung. American Journal of Respiratory and Critical Care Medicine 2019;200(9):1082-1083). This highly novel work showed that nicotine- containing e-cigarette

vapor preferentially acts through the TRPA1 receptor, as opposed to canonical nicotinic receptors, to induce mucociliary dysfunction both in vitro and in a large animal model (sheep) of e-cigarette exposure. The article has already been cited 56 times. Finally, a manuscript describing the adverse effects of propylene glycol (PG) and vegetable glycerin (VG) on mucociliary clearance and airway inflammation in vitro, in a sheep model in vivo, and in human volunteers is currently in preparation. The team has also published additional papers on smoke-induced changes in the airways and their signaling pathways and continue to collaborate with other institutions. Based on data obtained with James and Esther King funding, successful submission of an R01 application to the NIH was funded in September 2018. Future plans for this work include continuing to execute experiments examining the effects of e-cigarette vapors of JUUL and nicotine salts on the airway epithelium. Staff continue to explore the individual and synergistic effects of nicotine with flavorings on mucociliary clearance. Finally, clinical studies will continue to examine the effects of e-cigarette vapors containing nicotine and flavors on airway inflammation in human volunteers.

Follow on Funding: FAMRI, Novel anti-inflammatory therapy for smoke-associated chronic rhinosinusitis Federal Agency/Institute: FAMRI Grant Mechanism: CIA, Salathe

Collaborations: University of Alabama at Birmingham School of Medicine, Birmingham, AL. Stem cell therapy to treat smoking-induced airway inflammation. Researcher: Vamsee Raju, PhD.

University of Kansas Medical Center, Department of Population Health, Kansas City, KS. Changes in airway inflammation associated with replacing tobacco smoking with menthol JUUL vaping. Researcher: Nikki Nollen, PhD.

University of Kansas, Department of Pharmaceutical Chemistry. Deposition characteristics of E-cigarette vapors. Researcher: Cory Berkland, PhD.

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University of Bern, Institute of Anatomy, Bern, Switerland. Toxicity of e-cigarettes and IQOS tobacco heating systems. Researcher: Marianne Geiser, PhD.

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Patents: None at the time of reporting.

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

Principal Investigator: Vani Nath Simmons, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: Tobacco smoking is the leading preventable cause of cancer mortality. Pharmacotherapy and behavioral counseling have demonstrated independent and additive effects on smoking cessation rates; however, counseling is rarely chosen by smokers. Minimal self-help interventions, such as smoking cessation booklets, have very high potential reach, yet have shown low efficacy, with the exception of the extended self-help smoking interventions developed by this research team. Originally developed to prevent post-cessation relapse to smoking, these booklets titled, Forever Free, significantly reduced smoking relapse through two years of follow-up among individuals who had recently quit smoking and were extremely cost-effective. Based on its efficacy and cost-effectiveness, the team expanded the intervention to assist current smokers with initial smoking cessation as well as relapse prevention. The recently completed National Cancer Institute funded trial of this intervention titled, Stop Smoking for Good, revealed high efficacy through the 24-month follow-up, further 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. Although the current smoking prevalence among Hispanics (12.5%) is lower than non-Hispanic whites (18.1%), higher prevalence is observed among certain subgroups (e.g., Puerto Rican males, 35%). In Florida, the smoking prevalence among Hispanics (15.1%) is greater than the national prevalence, and it is higher among subgroups and within medically underserved communities. Prior work has demonstrated that Hispanic smokers face unique challenges such as lower awareness and acceptance of pharmacotherapies and less cessation assistance from health providers. This study goal is to expand the reach of our evidence-based, self-help intervention by developing and testing a Spanish-language version. This would represent an easily disseminable, low-cost intervention with significant public health impact for Hispanic smokers in Florida and elsewhere. The novel Spanish-language smoking cessation intervention will of course build upon content of the existing English-language intervention (based on cognitive behavioral theory) and relevant literature related to smoking among Hispanics. However, pivotal to the transcreation process will be the conduct of focus groups to enhance the cultural relevance and acceptability of the intervention among Hispanic smokers. Overall, this phase will allow staff to obtain reactions to the existing intervention materials (booklets and supportive stories) in terms of tone, character development and messaging design style. It addition, exploration of novel and culturally relevant smoking cessation themes and content for the intended audience will occur. Phase II - Once initial drafts of the Spanish-language components are developed using the data obtained from the focus groups, the team will begin learner verification iterations to further assess suitability for the intended audience. Phase III - The focus group data and learner verification processes will assist in achieving the goal, and guide the adaptation of the current intervention for Spanish-speaking smokers. At the most apparent surface level, language, photos, and graphics will reflect Hispanic culture. Additionally, content for the personal vignettes contained throughout the

ten booklets and the nine “My Story” supportive pamphlets will be derived from experiences and perceived benefits and barriers to quitting smoking as relayed during the focus groups as well as from the existent literature related to unique barriers and issues relevant for Hispanic smokers.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: A randomized controlled trial of a smoking cessation self-help intervention for Spanish-speaking Hispanic smokers: Study design and baseline Characteristics. Medina-Ramirez, P., Sutton, S. K., Martinez, U., Meade, C. D., Byrne, M. M., Brandon, K. O., Meltzer, L. R., Gonzales, F. M., Brandon, T. H., & Simmons, V. N. Contemporary Clinical Trials. August (2019).

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Patents: None at the time of reporting.

Live Like Bella Pediatric Cancer Research Initiative
Appendix V
Fiscal Year 2020-2021 Newly Awarded Active Grants
Funded Fiscal Year 2020-2021

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
21L01	University of Miami	Claes Wahlestedt, MD, PhD	$800,990	4/30/2026	No	No	No
21L02	University of Florida	Zhijian Qian, PhD	$100,000	10/31/2021	No	No	No
21L03	University of Florida	Mingyi Xie, PhD	$247,000	4/30/2024	No	No	No
21L04	H. Lee Moffitt Cancer Center	Uwe Rix, PhD	$247,000	4/30/2026	No	No	No
21L05	University of Florida	Jonathan Licht, MD	$247,000	4/30/2024	No	No	No
21L06	University of Florida	Lan Hoang-Minh, PhD	$247,000	4/30/2024	No	No	No
21L07	University of Miami	Paulo Pinheiro MD, PhD, MSc	$247,000	4/30/2024	No	No	No
21L08	University of Miami	Regina Graham, PhD	$247,000	6/30/2023	No	No	No
21L09	University of Florida	Raymond Mailhot, MD	$247,000	4/30/2024	No	No	No
21L10	Florida State University	Q.X. Amy Sang, PhD	$246,510	4/30/2024	No	No	No
21L11	University of Central Florida	Annette Khaled, PhD	$123,500	9/30/2022	No	No	No

1. **Grant #:** 21L01 Development of an IDE Submission for Drug Sensitivity Testing Platform for Pediatric Sarcoma Treatment Stratification

   Principal Investigator: Claes Wahlestedt, MD, PhD

   Organization: University of Miami

   Abstract: Sarcomas are cancers occuring in the bones and soft tissues with particularly high incidence rates in pediatric, adolescent and young adult patients. While young age is generally linked to a better prognosis, the pediatric population carries the highest burden from a sarcoma diagnosis. Not only does this patient population suffers from loss of limbs and pain but treatment-induced side effects such as loss of fertility, heart or lung problems and learning and development problems are very prevalent and debilitating. For many sarcomas, such as osteosarcoma and Ewing sarcoma among others, treatment has not changed significantly in decades and cure rates have plateaued. We have recently developed and tested an ex vivo drug sensitivity testing (DST) platform that allows the generation of personalized treatment plans, based on the sensitivity of the individual patient's tumor cells towards 215 FDA-approved compounds at the time of treatment. We believe that specifically pediatric patients will benefit from our treatment stratification approach and will therefore mainly concentrate on this patient population. Our DST platform does not depend on extensive histological or genomic tumor classification, both of which can have long turnover times that delay treatment start. Treatment stratification can be achieved in 10-14 days. Because all compounds are FDA-approved and are available on compassionate care, have well known toxicity profiles and treatment schedules

they are well perceived by prescribing physicians and health insurance companies. We have successfully evaluated our DST screen in sarcoma patients and were able to generate patient-specific treatment recommendations using surgical material. These pilot cohort allowed us to establish feasibility of our platform for the use in solid tumors and enforced the utility for clinical treatment stratification by proposing a treatment compound outside of the standard-of-care regimens. In order to further develop the DST screen for clinical routine use, we will develop an IDE application for submission to the FDA as a diagnostic device. We have submitted a request for risk determination to the FDA early this year for a randomized clinical investigating the DST platform. The FDA has classified the device as significant risk and requested an Investigational Device Exemption (IDE) submission prior to the start of the clinical trial. As part of these efforts, we will further improve the DST platform through the partial automation of the screening method and the development of a software package for the analysis of the DST data that will include modules for DST analysis, quality control, and data reporting, visualization and management. This automation will be evaluated for screen integration and quality and tested on archive samples in order to maintain DST screen quality. In addition to the further development of components of the screening method, we will develop the clinical and regulatory documents needed for IDE submission. This includes the clinical trial protocol needed for a randomized Phase III clinical trial, the manual of operations and procedures, standard operating procedures and consent documents on the clinical side and design controls (21 CFR 820.30) on the regulatory side. Approval of the IDE submission by the FDA will allow us to undertake a large multicenter clinical trial evaluating safety and clinical benefit of the DST platform and implement DST screening as part of the clinical routine for pediatric patients with sarcoma.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

2. **Grant #:** 21L02 FOXM1 as a Therapeutic Target for MLL-rearranged AMLs

Principal Investigator: Zhijian Qian, PhD

Organization: University of Florida

Abstract: Rearrangements of chromosome 11q23, which carries the mixed lineage leukemia (MLL) gene, occur in 18% of pediatric patients, and in up to 50% of infant acute myeloid leukemia (AML). AML patients who carry MLL-rearrangements have a very poor prognosis and are more resistant to traditional chemotherapy. To date, no effective targeted therapy is available for MLL-r AMLs. Accumulating evidence suggests that a small population of leukemia cells, called leukemia stem cells (LSCs), play an important role in the development and maintenance of AML, and cause leukemia relapse. This application aims to develop a novel strategy for a more effective treatment of MLL-r AMLs by selectively targeting the LSCs. Forkhead Box M1 (FOXM1) is a critical gene which regulates many biological processes in the mammalian cells. We found that FOXM1 was upregulated in MLL-r AML patients and that it was required for the maintenance of MLL-AF9-transformed LSCs. More importantly, we found that

MLL-AF9-transformed mouse or human LSCs were much more sensitive to deletion or inhibition of FOXM1 than the normal mouse and human hematopoietic stem progenitor cells (HSPCs). In this proposal, we will investigate how FOXM1 upregulation affects the functions of hematopoietic stem/progenitor cells and hematopoiesis in vivo; and decipher the molecular mechanisms underlying the role of FOXM1 in the maintenance of MLL-r LSCs. Finally, we will evaluate FOXM1 as a potential therapeutic target in the LSCs for pediatric MLL-r AML patients. This study will advance our understanding of the molecular biology of LSCs, and will likely lead to the development of novel therapeutic strategies to treat pediatric MLL-r AMLs by selectively targeting the LSCs.

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 #:** 21L03 Target RNAs Induce microRNA Degradation in Apoptotic T-cell Acute Lymphoblastic Leukemia Cells

Principal Investigator: Mingyi Xie, PhD

Organization: University of Florida

Abstract: Gene expression, the flow of genetic information from DNA to messenger RNA (mRNA) to protein, involves delicate regulation by a group of small RNAs properly named microRNAs. MicroRNAs can inhibit gene expression by binding to mRNAs and prevent them from being translated into proteins. MicroRNA levels in cancer cells are usually different from the microRNA levels in healthy cells, leading to differential expression of certain cancer-related genes. Controlling microRNA levels therefore offers a promising target for cancer treatment. Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer. Recently, we found that when T-cell ALL (T-ALL) cells are treated with dexamethasone, a steroid commonly used in leukemia chemotherapy, two highly related and pro-cancer microRNAs (miR-221/222) are degraded by their target mRNA (Bim). This is surprising because microRNAs usually control the levels of their targets, but not the other way around. This is also exciting because it reveals a new gene regulation mode carried out by the mRNAs and opens up strategies for cancer intervention. In this proposal, we first aim to understand how miR-221/222 degradation induced by Bim enhances dexamethasone sensitivity of T-ALL. Our second aim is to develop an innovative biochemical and computational protocol to globally identify sequences in different target mRNAs that can induce miRNA degradation in T-ALL. Collectively, our efforts will uncover a new mechanism of gene regulation, in which mRNAs counteract microRNAs. because resistance to dexamethasone is a serious limitation for pediatric T-ALL chemotherapy, elucidating the underlying mechanism of resistance may provide the basis for improving current therapeutic interventions. Given that we have discovered a potentially widespread occurrence of the mRNA-induced microRNA degradation pathway, identifying the mRNAs that can degrade miRNAs would help develop new therapies to combat pediatric T-ALL and other 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.

4. **Grant #:** 21L04 Characterization of PARP16 as a Novel Target in Ewing's Sarcoma

Principal Investigator: Uwe Rix, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: Ewing's sarcoma (EWS) is a pediatric cancer with dismal prognosis and a critical need for novel therapies. The objective of this proposal is to determine the functional relevance of the non-canonical mono-ADP-ribosyltransferase poly adenosine diphosphate-ribose polymerase (PARP)16 and its underlying molecular mechanism in EWS. Our central hypothesis is that PARP16, alone and in conjunction with PARP1, constitutes a novel therapeutic target in EWS. We have identified PARP16 as a unique target of the PARP1 inhibitor (PARPi) talazoparib that is not hit by other PARPis, but makes an important contribution to talazoparib's overall mechanism of action (MoA) in EWS cells. However, talazoparib is only a partial inhibitor of PARP16 at clinically tolerated concentrations and the biological function of PARP16 and its relationship to PARP1 in EWS is completely unknown. Little is understood about PARP16. Prior reports have suggested a role in the unfolded protein response (UPR) and endoplasmic reticulum (ER) stress signaling, but these studies were performed in HeLa or non-mammalian cells, whereas our preliminary data suggest that ER stress induction is not affected by PARP16 in EWS cells. The rationale for this project is that a thorough understanding of PARP16's MoA is required for designing suitable assays and functional cellular readouts that are critical for the successful development of more potent inhibitors of PARP16 or additional downstream targets with translational utility in EWS. Specific aims: Aim 1. Determine the cellular phenotypes affected by PARP16 and it's in vivo functional relevance in EWS. Using a panel of EWS cell lines with defined sensitivities to PARP16 targeting on cell viability, we will determine the effects of PARP16 silencing and overexpression on apoptosis, cell cycle and induction and repair of DNA damage, as well as on EWS-FLI1 and combinations with standard drugs. To evaluate crosstalk of PARP16 with PARP1, we will add PARPis with no cross-selectivity for PARP16. To determine the functional relevance of PARP16 for EWS in vivo, we will perform mouse xenograft experiments with inducible PARP16 shRNA in the absence and presence of a PARPi. Tumor markers for proliferation, apoptosis and DNA damage will be determined by immunohistochemistry (IHC) upon tumor harvest. Aim 2. Elucidate the molecular mechanisms that underlie the vulnerability of EWS cells to PARP16 targeting. Using mass spectrometry-based protein-protein interaction and ADP-ribosylation proteomics upon overexpression of PARP16, we will identify interaction partners and substrates of PARP16 in EWS cells in the absence and presence of PARP1- specific inhibitors. We will also determine changes on total protein expression upon induced silencing of PARP16. Integrated analysis will generate a comprehensive view of PARP16 action and nominate proteins for functional validation. Key candidate effectors will be validated using coimmunoprecipitation, immunoblotting, CRISPR, RNAi, pharmacological inhibition and rescue experiments, as well as IHC in patient specimens.

This project will provide a comprehensive, proteome-wide understanding of the molecular mechanisms of EWS vulnerability towards targeting of PARP16, elucidate its crosstalk with PARP1 and define its relevance as a novel target for Ewing's sarcoma. This work is expected to pave the way for future development of PARP16-selective or dual PARP1/16 inhibitors as new drugs for EWS, for which novel therapeutic modalities are urgently needed.

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 #:** 21L05 NSD2 Mutation as Driver of Brain Invasion in Acute Lymphoblastic Leukemia

Principal Investigator: Jonathan Licht, MD

Organization: University of Florida

Abstract: Our laboratory studies the NSD2 methyltransferase which undergoes an activating mutation in ~10% of cases of relapsed pediatric acute lymphoblastic leukemia (ALL). Accumulation of the mutation at relapse suggested that it causes drug resistance. ALL often relapses in the central nervous system and treatment of the brain and spinal cord causes neuronal damage and may impair intellectual development. Identification of new and potentially less toxic approaches to CNS disease remains important for the treatment of ALL. Preliminary data: Through our Live Like Bella grant we showed that Nuclear Receptor Binding SET Domain Protein 2 (NSD2) E1099K created resistance to glucocorticoids in ALL due to suppression of glucocorticoid receptor (GR) levels. Resistance was reversed by pre-treatment of NSD2 mutant ALL cells (including patient derived xenografts) with Enhancer Of Zeste 2 Polycomb Repressive Complex 2 Subunit (EZH2) inhibitors, reactivating expression of the GR, allowing glucocorticoids to induce proapoptotic genes. This extended lifespan in mice injected with ALL cells but did not eradicate the tumors, suggesting other NSD2 therapies are required. However, despite extensive efforts, NSD2 remains undruggable, so other approaches, such as targeting the genes regulated by NSD2 are needed. NSD2 mutant ALL cells had aggressive growth properties including prominent central nervous system (CNS) invasion in mice. Removal of the NSD2 mutation by gene editing slowed cell growth and prevented extensive brain infiltration by tumor cells. NSD2 E1099K upregulated genes in pathways of cell adhesion, signaling, axon guidance and neuronal development, the latter not normally expressed in blood cells. This aberrant gene expression may explain the tendency of NSD2 mutant cells to traffic to the brain. To extend these findings we will: Aim 1: Determine genes that promote CNS infiltration of NSD2 E1099K cells. We hypothesize that genes and pathways downstream of NSD2 drive cell growth and CNS invasion. These genes may represent new therapeutic targets for NSD2 mutant and other forms of ALL and could yield more general approaches to CNS relapse. We will functionally validate the importance of these genes. Using CRISPR, we will disrupt genes activated by mutant NSD2 and screen for effects on cell migration, invasion and adhesion in cell culture. A screen conducted in mice will identify genes involved in infiltration of the CNS and other organs. These studies will be corroborated by CRISPRactivation experiments to

aberrantly activate expression of NSD2 mutant targets in wild-type ALL. Genes that simulate ALL cell migration and adhesion in cell culture and required for meningeal and brain parenchymal infiltration in mice represent potential new therapeutic targets. Aim 2: Determine gene expression changes in NSD2 E1099K cells upon exposure to brain microenvironment cells. Multiple groups showed that upon adhesion of ALL tumor cells to cells surrounding the brain such as endothelial and meningeal cells, gene expression changes are induced in the leukemia cells that facilitate cell growth and invasion. We hypothesize that NSD2 mutant ALL cells have an altered or enhanced response to supporting endothelial cells of the brain. We will identify these gene expression changes and ablate them by knockout or knockdown strategies to validate additional target genes and pathways to prevent CNS invasion. For both aims, drugs against the pathways we identify, critical for NSD2 mutant cell behavior, will be tested in cell culture and in mice harboring human ALL cells.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

6. **Grant #:** 21L06 Combination Immunotherapy for Pediatric Brain Tumors

Principal Investigator: Lan Hoang-Minh, PhD

Organization: University of Florida

Abstract: Malignant brain tumors are the most frequent cause of cancer-related deaths in children. Despite aggressive and highly toxic therapies including surgery, radiation and high-dose chemotherapy, almost half the children diagnosed with the most common malignant brain tumors, high-grade glioma and medulloblastoma, will die from recurrent disease. Survivors are often left with severe and lifelong treatment-associated neurological deficits. The development of more effective and tumor-specific therapies that will not add further toxicity to existing treatments is crucial in improving clinical outcomes for children affected by those aggressive cancers. Immunotherapy targeting tumor-specific molecules expressed by brain tumors has the potential of meeting this clear and urgent public health need. Adoptive T cell therapy (ACT) involves using the patients' own immune cells, called T cells, to specifically destroy their brain tumor. ACT has become the immunotherapy with the highest curative potential for patients with advanced, invasive, and recurrent malignant diseases, such as metastatic melanoma. For pediatric brain tumors, an ACT platform employing those T cells has proven to be more effective than standard therapies in preclinical and clinical studies conducted at our center. However, complete remissions have not been achieved for most patients. One of the contributing factors might be immunosuppression, including the upregulation of molecular brakes or immune checkpoints, particularly Programmed cell death protein 1 (PD-1), on transferred ACT cells and patients' endogenous T cells. Immune checkpoint inhibitors against PD-1 have been used successfully in the clinic against multiple cancers, particularly when administered before surgical tumor removal. Notably, recent early phase clinical trials have shown increased survival and objective responses in adult high-grade glioma patients who received PD-1 blockade before

resection of their tumor. However, the exact mechanisms underlying these effects are still unclear, and this approach has never been investigated for pediatric brain tumors. Our preliminary studies show that anti-PD-1 treatment before surgery increases the recruitment of T cells at recurrent tumor sites and enhances survival in a new preclinical resection model of recurrent glioma that we have established. Our hypothesis is that targeting PD-1 will enhance the effectiveness of ACT against pediatric brain tumors. The goals of this proposal are to investigate the effects and underlying mechanisms of anti-PD-1 treatment administered before or after tumor resection; examine the efficacy of ACT using T cells devoid of PD-1 after CRISPR-Cas9 genome editing; and test the effects of combining those strategies in preclinical models of pediatric high-grade glioma and medulloblastoma. ACT T cells will be monitored using magnetic particle imaging, a novel non-invasive imaging technology developed in collaboration with Dr. Carlos Rinaldi in the University of Florida College of Engineering. To optimize translation into clinical trials, our in vivo preclinical studies will be designed and conducted in consultation with our collaborators Drs. Duane Mitchell and Sri Gururangan, principal investigators of pediatric neuro-oncology clinical trials currently conducted at the University of Florida. Our findings will be critical in applying novel, more effective immunotherapy approaches for pediatric patients diagnosed with malignant brain tumors and other childhood cancers.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

7. **Grant #:** 21L07 The Role of Prenatal Exposures and Specific Ethnicity on Childhood Cancer Disparities in Florida

Principal Investigator: Paulo Pinheiro MD, PhD, MSc

Organization: University of Miami

Abstract: Research in childhood cancer disparities has lagged behind the surge of racial-ethnic diversity that characterizes the country's younger generations. Hispanic children represent the fastest growing and largest minority in the US, yet their heterogeneous genetic backgrounds are largely ignored in oncology research. Acute lymphoblastic leukemia (ALL), the most common pediatric malignancy, inequitably burdens Hispanic children who have the highest incidence and mortality rates. The etiology of racial and ethnic disparities is largely unknown. In ALL, genome-wide association studies have identified high-risk alleles among Hispanics. In other malignancies, perinatal factors (e.g., assisted conception, birth defects) have been associated with cancer risk. However, the differential impact of such factors across the full spectrum of race-ethnicity has not been studied. Finally, survival disparities in pediatric oncology are evident with Hispanic and Black children faring worse than Whites. The variability of clinical factors impacting cancer prognosis between detailed racial-ethnic groups has yet to be evaluated. Currently, national data on specific ethnic patterns among Cubans, Puerto Ricans, Mexicans, Dominican South and Central American (CPMDSCA) children do not exist. To fill in these

important knowledge gaps, we will study Florida's pediatric cancer population, leveraging its unique diversity. All CPMDSCA and the Black Caribbean group have populations larger than 500,000 (exception Dominicans: 300,000) in Florida. In an initiative whose detail can only be achieved at the state (rather than national) level, we will link all Florida Cancer Data System pediatric/adolescent cancers from 0-24 years old diagnosed between 2000-2018 to the state birth certificates from 1980-2018 (demographic and clinical data). This unique linkage will enable us to study previously unknown ancestral characteristics (cancer registry data on children's ethnicity is highly incomplete and is available from birth certificates) and unique perinatal characteristics in relation to cancer incidence and outcomes. Based on this linkage and on reviews of electronic medical records from two South Florida institutions (Sylvester Comprehensive Cancer Center and Jackson Memorial Hospital) we propose the following aims. Aim 1. Examine incidence and survival disparities for all detailed Hispanic (CPMDSCA) and non-Hispanic Black subgroups (African American, Afro-Caribbean) for childhood cancers combined and for ALL, acute myeloid leukemia, brain tumors, non-Hodgkin lymphoma, bone and soft tissue sarcoma, etc. Aim 2. Examine the effect of use of assisted contraception and other prenatal risk factors for childhood cancers in Hispanic and Black children as compared to Whites and correlate findings with those of Aim 1. Aim 3. Review clinical records of children with ALL to examine how clinical prognostic factors such as white blood cell count at diagnosis, cytogenetics, minimal residual disease, and treatment information can explain ALL survival disparities observed in Aim 1. In an era of increasingly targeted and individualized therapies in pediatric oncology, it is fundamental to understand the impact of genetic and perinatal factors across the spectrum of racial and ethnic diversity. By better understanding childhood cancer, its risk and prognostic factors, our proposed study will be uniquely valuable to fight childhood cancer in the growing multi-racial multi-ethnic pediatric population.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

8. **Grant #:** 21L08 Carbon Dot Derivative for Bimodal Imaging and Targeted Drug Delivery to Pediatric High-Grade Gliomas

Principal Investigator: Regina Graham, PhD

Organization: University of Miami

Abstract: Malignant brain tumors remain a significant cause of morbidity and mortality in children and adults. In fact, brain tumors are now the number one cause of cancer related deaths in children. For the children that do survive the adverse consequences of current treatment regimens are severe, with significant developmental and neurocognitive deficits and an increased risk of additional cancers. Due to the invasive nature or location of tumors a complete surgical resection is not feasible. Thus, successful treatment depends on delivery of effective anti-cancer agents to the brain tumor. However, the blood brain barrier (BBB) prevents most anti-cancer drugs from reaching the brain tumor at therapeutic levels. Another challenge is

brain tumor heterogeneity which refers to the phenomenon that not all brain tumor cells within a tumor are identical and demonstrate differences in gene expression and drug susceptibility. To overcome these obstacles, we propose to develop a novel drug delivery system (DDS) which penetrates the BBB and selectively delivers chemotherapy to brain tumor cells using non-toxic Carbon-dots (C-dots) as the nanocarrier. C-dots are small carbon nanoparticles with excellent optical properties, high biocompatibility and low cost, making them a promising candidate for the development of DDS. Our research demonstrates that highly fluorescent C-dots and C-dot derivatives such as carbon nitride dots (CN-dots) are 1. non-toxic and able to cross the BBB; 2. can be functionalized with tumor-specific targeting ligands to increase specificity and reduce adverse side effects and; 3. can be functionalized to deliver multiple chemotherapies thereby increasing tumor cell killing and reducing the development of drug resistance. Further, we have shown the highly fluorescent CN-dots and are preferentially taken up by brain tumor cells and when conjugated to chemotherapy, selectively kill brain tumor cells. The ability to image the DDS in real-time can provide valuable information regarding bio distribution, tumor localization, pharmacokinetics and toxicity which is necessary for clinical integration thus a DDS should be designed for imaging. Therefore, the goal of this research is to develop a tumor-targeted gadolinium doped CNdot DDS which combines two imaging modalities: magnetic resonance imaging (MRI) for noninvasive imaging and fluorescence for identifying the DDS containing cells within the tissue ex vivo. To accomplish this, two major aims are proposed. In aim 1 we will synthesize and characterize gadolinium embedded CN-dots (Gd@CN-dots). We will thoroughly investigate the intrinsic properties of Gd@CN-dots by studying both the surface and the inner core properties of the CN-dot derivative and relate this to tumor cell targeting, fluorescence imaging, biocompatibility and MRI contrast ability. In aim 2 we will synthesize and characterize drug loaded Gd@CN-dots. Further, we will explore the potential of increasing tumor specificity by conjugating ligands which target receptors preferentially expressed on brain tumor cells. The ability of drug loaded Gd@CN-dots to target and kill tumor cells will be investigated first in vitro against a panel of pediatric high-grade glioma cell lines and then in vivo using mouse models of pediatric glioblastoma and diffuse intrinsic pontine glioma. Successful development of our C-dot DDS could significantly improve treatment outcomes while simultaneously improving the patient's quality of life

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 #:** 21L09 Measuring the Effects of Brain Radiotherapy and Tumor on Scholastic Outcome

Principal Investigator: Raymond Mailhot, MD

Organization: University of Florida

Abstract: Compared to conventional x-ray-based therapy (XRT), proton radiotherapy has the potential to mitigate long-term toxicity. As a state-of-the-art approach to brain radiotherapy, proton therapy can reduce the amount of healthy tissue exposed to radiation. Both brain tumors

and radiotherapy can damage children's cognitive abilities, as has been shown in prospective postradiotherapy studies measuring IQ. Recent studies have shown that proton therapy controls cancer as well as XRT does, without decreasing IQ. What is yet unexplored is the relationship between brain tumors, radiotherapy (XRT and proton therapy) and scholastic success. The Florida Department of Education administers statewide assessments on student performance. Beginning in 2001, the Florida Comprehensive Assessment Test (FCAT) was expanded to grades three through ten, allowing for the calculation of annual student learning gains. In 2003, passing the Grade ten Reading and Mathematics FCAT became a requirement for high school graduation. Since 2019, physicians at UF Health Proton Therapy Institute have treated approximately 900 children with brain tumors, reflecting the largest experience in pediatric proton therapy worldwide. By focusing on our Floridian patients, we are well-poised to study the relationship between pediatric brain tumors, proton therapy and scholastic success within our community. Specific Aim 1: Determine the change in scholastic ability, as measured by FCAT scores, in the years leading to cancer diagnosis among local children with brain tumors treated at UF. Hypothesis 1: Compared to the general population, children with brain tumors will have lower gains in scholastic ability in the years leading up to a cancer diagnosis due to the adverse impact of a growing tumor on neurologic development. Specific Aim 2: Determine the change in scholastic ability, as measured by FCAT scores, after treatment among local children with brain tumors treated with radiotherapy and whether the magnitude in change differs by race or ethnicity. Hypothesis 2: Children treated with proton therapy will have minimal negative impact on scholastic gains after treatment. Methods: We will review the medical records of local pediatric patients who underwent brain radiotherapy (proton therapy and/or XRT) at UF under our IRB-approved study (UFJ-2014-128). For each child, we will determine the amount of brain irradiated and obtain FCAT/FCAT 2.0 scores across several domains, including reading and mathematics. To minimize confounding from medical covariates, children will be stratified by surgery, receipt of chemotherapy and presence of hydrocephalus. To minimize confounding on scholastic gains influenced by socioeconomic status, we will incorporate median income per the patients' 9-digit zip code. We will use longitudinal analysis to detect any statistically significant negative trend in FCAT scores in the years leading up to a child's diagnosis. We will also compare changes in test scores among children with brain tumors and Floridian students overall at similar time points to serve as a "healthy" reference. Finally, we will determine if any disparities in scholastic outcomes exist in our pediatric survivors by race or ethnicity. Relevance: This proposal will fulfill the Live Like Bella initiatives to improve our understanding of (and outcomes with) radiotherapy and its effects on cognition and to identify disparities in scholastic outcome among children with brain tumors

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

10. **Grant #:** 21L10 Modeling Human Pediatric Brain Tumor Microenvironment

Principal Investigator: Q.X. Amy Sang, PhD

Organization: Florida State University

Abstract: Human pediatric brain cancer is one of the most common and lethal types of solid tumors in children. Primary childhood tumors include low-grade and high-grade gliomas, medulloblastoma and atypical teratoid/rhabdoid tumor (ATRT). Brain tumor microenvironment (TME) is enriched with tumor associated astrocytes, microglia, macrophages, neutrophils, T cells and endothelial cells. However, the complex interactions between brain cancer cells and these TME cells are not well-understood. TME may play an important role in tumor progression, therapeutic resistance and immune suppression. This project will build a novel 3-dimensional (3-D) human pediatric brain cancer coculture model using human ATRT, medulloblastoma, and glioma cell lines incorporating tumor microenvironment astrocytes and microglia in different 3-D compartments. Based on scientific literature, we propose the hypotheses that tumor associated astrocytes may produce interleukin-4 (IL- 4) and other cytokines to polarize tumor associated microglia/macrophages (TAM), and then TAM will produce insulin-like growth factor 1 (IGF1) and other cytokines and growth factors, which will promote tumor progression and invasion. The three specific aims are: Specific Aim 1: To characterize human astrocytes and microglia-like cells derived from human induced pluripotent stem cells (iPSCs). Specific Aim 2: To test the hypothesis that astrocytes and microglia may promote cancer cell proliferation, migration and invasion through paracrine interactions. A modified Boyden Chamber transwell system will be built to model tumor and stromal interactions and to evaluate paracrine interactions between pediatric cancer cells and TME cells. The rates of cancer cell proliferation, migration and invasion will be measured using the modified Boyden Chamber and the Millicell Microfluidic Migration Device. Selective human pediatric ATRT, medulloblastoma and glioma cell lines will be purchased from the American Type Culture Collection (ATCC). Specific Aim 3: To test the hypothesis that tumor associated astrocytes and microglia may promote chemoresistance. The novel 3-D cell culture system and the Microfluidic Migration Device will be utilized to grow the cancer cells and compare the chemotherapy effectiveness in the presence and absence of TME cells. Specific Aim 4: To test the hypothesis that the immune suppressive TME can be overcome by human natural killer (NK) cells and gamma delta T cells derived from human iPSCs. Understanding of the complex paracrine interactions between human pediatric brain cancer cells and TME cells will help us to design novel therapeutic strategies to target the cancer cells and the TME cells. Modified NK cells and gamma delta T cells may become future cell-based immunotherapeutics targeting human pediatric brain tumors.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

11. **Grant #:** 21L11 Evaluating Chaperonin-Containing TCP1 for the Screening of Pediatric Cancers

Principal Investigator: Annette Khaled, PhD

Organization: University of Central Florida

Abstract: Over 11,000 children in the USA will be diagnosed with cancer in 2020. While this accounts for about 1% of all cancers, this number is increasing. Screening for pediatric cancer is difficult because there are no routine tests for children that are not at risk. It is only when an abnormal lump or tumor is found that biopsies are performed, which are especially difficult to do in children. Biopsies are invasive procedures that may cause damage and carry the risk of infection. Imaging tests, like x-rays, also have associated risks with radiation exposure. To address the need for a pediatric cancer screen that is safe and can be performed routinely on children, a liquid biopsy approach is promising. Typically, liquid biopsy involves drawing a small amount of blood and determining if there are tumor cells or DNA present. Unlike tissue biopsies, the cells and molecules detected in blood are representative of the most aggressive parts of a tumor. To date there are no liquid biopsy protocols for pediatric cancer screening. Part of the reason is that, while pediatric cancers may shed tumor cells or DNA, these harbor few genomic aberrations; hence, there are few biomarkers known that can be used to capture circulating tumor cells (CTCs) in blood. As example, the CellSearch System (CSS) is the only FDA approved technology for the detection of CTCs in some adult cancers. Yet this protocol for assessment of CTCs in blood involves only counting cells, not assessing the potential for tumor growth, which is important for pediatric cancers. To address this unmet medical need, our lab developed a CTC detection protocol that centers on a novel biomarker – a protein-folding complex called CCT or Chaperonin-Containing TCP1. CCT folds many of the proteins involved in carcinogenesis, including those identified in pediatric cancers like MYCN and mutantTP53. We found that expression of the chaperonin is amplified in many adult cancers as compared to healthy tissue and is a universal factor frequently upregulated to meet the demands for increased protein folding as a result of oncogenesis. CCT is not cancer type specific. Which makes the chaperonin an attractive candidate for detection of pediatric cancer CTCs. Our protocol for CTCs is more sensitive than the conventional CSS protocol and can identify CTCs that are missed by the CSS as well as determine the tumorigenic potential of the captured CTCs. These features are unique to our liquid biopsy platform. The objective of our application is to determine whether CCT can be used to detect pediatric cancers and determine which cancer type would most benefit. As a first step, we examined existing genomic databases and found that alterations in the genes encoding the chaperonin occur in pediatric bone cancers (>17%) and peripheral nervous system cancers (>11%) as well as others. We hypothesize that increased expression of the chaperonin at the genomic and/or protein level occur in pediatric cancers to support the oncogenic process. To investigate this, we propose, in two aims, to examine CCT expression in pediatric cancer tissues and CTCs in blood and then, using pediatric cancer cell lines, examine the dynamics of CCT expression in select childhood cancers. Through these studies, we will validate the chaperonin as essential in pediatric cancers and advance our liquid biopsy approach for screening pediatric patients as well as provide a non-invasive way to monitor treatment outcomes and cancer recurrence.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Live Like Bella Pediatric Cancer Research Initiative
Appendix W
Fiscal Year 2020-2021 Active Grants
Funding Fiscal Year 2019-2020

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
20L01	Florida State University	Akash Gunjan, PhD	$219,138	5/31/2023	Yes	No	No
20L02	H. Lee Moffitt Cancer Center	Damon Reed, MD	$787,272	4/30/2024	No	Yes	No
20L03	H. Lee Moffitt Cancer Center	Bijal Shah, MD, MS	$219,138	4/30/2023	No	No	No
20L04	Nemours Children's Hospital	Tamarah J. Westmoreland, MD, PhD	$219,138	4/30/2023	No	No	No
20L05	University of Central Florida	Cristina M. Fernandez-Valle, PhD	$218,572	5/31/2023	No	No	No
20L06	University of Central Florida	Li-Mei Chen, MD, PhD	$109,569	5/31/2023	No	Yes	No
20L07	University of Florida	Elias Sayour, MD, PhD	$788,897	5/31/2023	No	Yes	No
20L08	University of Florida	Coy D. Heldermon, MD, PhD.	$219,318	5/31/2023	No	No	No
20L09	University of Miami	Julio C. Barredo, MD	$219,138	5/31/2022	No	Yes	No

1. **Grant #:** 20L01 Targeting Wild-Type Isocitrate Dehydrogenase (IDH) Enzymes for Treating Lethal Pediatric Diffuse Intrinsic Pontine Gliomas (DIPG) Driven by Histone H3.3 K27M Mutations

Principal Investigator: Akash Gunjan, PhD

Organization: Florida State University

Abstract: DNA is our genetic material, and it regulates all aspects of human health, including diseases such as cancer. Histones are proteins that bind our DNA and package it into chromosomes, thus determining which genes are turned on and off, and when. Mutations in histone protein H3.3 drive the formation of lethal brain tumors known as glioblastomas, as well as highly disfiguring tumors such as chondroblastomas (cartilage tumors) and large cell tumors of the bone in children and young adults. How H3.3 mutations drive these tumors primarily in children is not yet understood. The H3.3 K27M mutation results in the change of the amino acid lysine (K) to a methionine (M) at position 27 in the H3.3 protein. This mutation is responsible for at least 80% of all cases of the invariably fatal Diffuse Intrinsic Pontine Glioma (DIPG), an aggressive high grade tumor that involves the brain stem. Because the brain stem controls basic body functions including breathing and heart rate, surgical removal DIPG tumors is mostly impossible. In addition, there are no approved chemotherapeutics to treat DIPG patients. DIPG's median age for diagnosis is six-seven years and the median survival rate is less than 9 months following diagnosis. Hence, DIPG is currently a heartbreaking and devastating cancer for patients and their families, with no hope in sight.

Using patient derived tumor cells, the research staff has been studying the H3.3 K27M mutant DIPG tumors with the goal of developing targeted therapeutics for eliminating this disease. So

far, they have discovered that the H3.3 K27M mutant protein binds to Isocitrate Dehydrogenase 1 (IDH1) enzyme and enhances its activity both in vitro and in the DIPG tumor cells, resulting in high levels of alpha-ketoglutarate (α-KG). High levels of α-KG in turn drive the excessive removal of a chemical modification known as “methylation” from DNA and histone proteins, resulting in very low levels of methylation in the H3.3 K27M mutant DIPG cells. Appropriate levels of methylation are crucial for proper gene expression and aberrant levels can drive cancer. More importantly, the low methylation levels can serve as a molecular “Achilles heel” for these tumor cells since the methylation levels can be potentially increased by blocking the enzymes that normally remove methylation. This can be achieved using a class of drugs known as IDH1 inhibitors, of which Ivosidenib was recently approved by the Food and Drug Administration (FDA). The in vitro data so far shows that in combination with standard radiation therapy, this drug can be used to specifically kill the H3.3 K27M DIPG tumor cells, while mostly sparing the normal cells. The research staff is now laying the groundwork now for testing the effectiveness of this therapeutic strategy in eradicating human patient derived DIPG tumors implanted in mice.

If successful, this project will lead to the development of an effective treatment for DIPG tumors, thus bringing hope to the affected children and their families, which is aligned with the research priority of the Live Like Bella Pediatric Cancer Research Initiative.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of this reporting.

Journals: None at the time of reporting.

Patents: Gunjan, A. “Selective treatment of cancers having histone H3 mutations or aberrant levels of DNA or histone methylation, acetylation or defects in homologous recombination.” (Application-17/303,457, filed on May 28, 2021 by Florida State University).

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

Principal Investigator: Damon Reed, MD

Organization: H. Lee Moffitt Cancer Center

Abstract: We have activated the trial here at Moffitt Cancer Center and have sent out information to all affiliate sites throughout the sunshine project network towards opening this study throughout Florida on this grant and throughout the nation with other foundation funds including from the National Pediatric Cancer Foundation.

Follow on Funding: None at the time of reporting.

Collaborations: Investigators at sites throughout Florida in the existing Sunshine Project consortium can open this trial and have been made aware that the trial is available.

Journals: Reed DR, Pressley M, Fridley BL, Hayashi M, Isakoff M, Loeb DM, Makanji R, Metts JL, Roberts RD, Trucco M, Wagner LM, Alexandrow M, Gatenby RA, and Brown JS. An

Evolutionary Framework for Treating Pediatric Sarcomas. Cancer. (2020). doi: 10.1002/cncr.32777. [Epub ahead of print] PubMed PMID: 32176331.

Patents: None at the time of reporting.

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

Principal Investigator: Bijal Shah, MD, MS

Organization: H. Lee Moffitt Cancer Center

Abstract: Health impact to Floridians

In this project, research project staff are investigating the link between aging and lung cancer, by testing the hypothesis that aging-induced rewiring of macrophage metabolism promotes both NAD+ biosynthesis in lung epithelial cells and a proinflammatory state in macrophages, thereby conferring an increased risk for lung cancer. This work holds great promise for identifying new and actionable therapeutic targets for treating lung cancers that do not respond to conventional therapies. In our aims, project staff will evaluate therapeutics that target the rewiring of NAD+ metabolism in both the tumor cell and macrophage populations.

Aim 1: Define the contribution of age-induced QA to lung NAD+ pools and carcinogenesis
The goal of this aim is to evaluate if QA, an age-induced NAD+ precursor, significantly contributes to the NAD+ pools of NSCLC and therefore affects the lung tumorigenic process. Staff will evaluate this possibility by performing tracing analysis in vivo and in vitro, by testing if increase QA levels affects lung tumorigenesis in mouse models and by using genetic tools to knockdown QA catabolic enzyme, QPRT.
Over this short period of funding staff have started to optimize the models and tools to perform these analyses. First, staff have optimized procedures to follow NAD+ metabolism in live mice and will soon apply these procedures to mice with tumors. Second, staff have optimized their tools to study NAD+ metabolic enzymes in lung cancer cells. Finally, staff have begun optimizing the treatment of mice with the NAD+ precursor QA.

AIM 2: Define the contribution of aged macrophage NAD+ decline to lung inflammation and carcinogenesis
Aging-induced defects in metabolism lower NAD+ levels in circulatory macrophages, leading to a proinflammatory state. However, the role of aged lung resident macrophages in lung tumor inflammation and tumor formation has not been established. The goal of this aim is to understand whether aging changes the metabolism of macrophages, thereby predesposing to lung cancer via increased inflammation.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of this reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

4. **Grant #:** 20L04 Zika Virus Mediated Lysis of CD24 positive neuroblastoma

Principal Investigator: Tamarah J. Westmoreland, MD, PhD

Organization: Nemours Children's Hospital

Abstract: The purpose of this grant is to evaluate Zika viral therapy in neuroblastoma that is resistant to the chemotherapy agent, Cisplatin. Chemoresistance is a challenging problem in neuroblastoma where approximately 50% of children with this tumor will relapse. When the children develop a relapse, their overall survival is poor. Our research has focused on novel therapies for chemoresistant neuroblastoma. This research has progressed well and has completed Aim 1 of the grant. Aim 1 is focused on the in vitro study of Zika viral therapy on Cisplatin-resistant neuroblastoma. Initially, the cell lines IMR 32 (advanced neuroblastoma) and SK-N-AS (recurrent neuroblastoma) were studied as our laboratory has published on these in the past. Zika viral treatment of IMR 32 results in greater than 95% cell death whereas the SK-N-AS cell line is resistant. CD24, a cell surface protein, is required for the Zika viral sensitivity. SK-N-AS does not have significant expression of CD24. When both of these cell lines were made Cisplatin resistant and treated with Zika virus, both cell lines were sensitive to Zika virus with greater than 95% cell death. In further examination, CD 24 expression was noted at signifiant levels in both cell lines. Cisplatin resistance had induced CD24 expression in the SK-N-AS cell line which made it sensitive to Zika virus. This is a significant finding and opens the door to Zika viral treatment to chemoresistant neuroblastoma whether CD24 is expressed at presentation or not. Following through Aim 1, additional paired pre- and post- treatment neuroblastoma cell lines were treated with Zika virus. Two of the neuroblastoma cell lines (SMS-KAN and SMS-KCN) were developed from primary pelvic neuroblastoma tumors, and they are paired with their bone marrow relapse counterparts (SMS-KANR and SMS-KCNR). An additional metastatic at the time of diagnosis neuroblastoma cell line (SK-N-Be 1) was also utilized with its relapse counterpart (SK-N-BE 2). All of these cell lines were grown and created to be resistant to Cisplatin. This was a challenging portion of the study to induce resistant tumors to recreate the resistance cells that contribute to the relapsed tumors. None of the patients from whom the cell lines were created were treated with Cisplatin in the past. All of the cell lines, whether primary or relapsed, were sensitive to Zika virus. All of the cell lines had greater than 90% cell death with a single treatment of Zika virus as seen on microscopic examination. All of the resistant neuroblastoma cell lines expressed the cell surface protein, CD24, which is involved in Zika viral entry. Overall, these findings are excellent because they reveal a novel treatment for chemoresistant neuroblastoma that is not available currently. This will be a direct impact on those Floridian children who develop chemoresistant neuroblastoma. These results will be now used to advance Aim 2 of the grant, which is to grow these chemoresistant neuroblastoma cell lines in mice and treat with Zika virus. These in vitro results are very encouraging for the success of the murine studies.

Follow on Funding: None at the time of reporting.

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

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Cristina M. Fernandez-Valle, PhD

Organization: University of Central Florida

Abstract: The purpose of this research is to identify cell surface markers which could be used to detect circulating tumor cells (CTCs) produced by malignant peripheral nerve sheath tumors (MPNSTs) in patients with Neurofibromatosis Type 1 (NF1). NF1 is a neurocutaneous syndrome that can produce tumors in the Central and Peripheral Nervous Systems, the most common of which are neurofibromas. Plexiform neurofibromas can allow MPNSTs to develop within the existing tumors, typically derived from the nerve sheath. No simple diagnostic test is available for MPNST detection. CELLSEARCH has developed a circulating tumor cell test, which can capture and isolate EpCAM positive circulating cells in low concentrations in a sample of patient blood for detection of this specific indicator of malignant cancer.

CELLSEARCH also has a number of other cell surface receptors that can be used and/or custom-made products that can be developed for detection of CTCs. Once cells are isolated from patient blood with a specific cell surface receptor, they are stained for secondary markers and exclusion markers to confirm the identy of the captured cell.

The goal of the first year of this award was to develop a MPNST cell capture assay. We have succeeded in identifying a common cell surface marker and a secondary marker expressed in multiple human MPNST lines. The progress this year includes: The team has obtained approvals from the Institutional Review Board to conduct work in years one, two and three. Next, the investigators are negotiating a Material Transfer Award with three hospitals (Baptist Hospital, Nicklaus Children's Hospital, Arnold Palmer Hospital for Children) needed to conduct work in years two and three. We have also optimized detection of epidermal growth factor receptor as primary marker and S100B as secondary marker by flow cytometry for cell capture (rather than EpCAM), and characterized expression of multiple markers of interest in four of five MPNST cell lines present in laboratory (SNF96.2, ST88, SNF94.3, SNF02.2, STS-26T). We have acquired seven new MPNST cell lines (NMS2, NMS2-PC, S462, NSF1, Hs-Sch2, Hs-PSS, 2XSB) under a Material Transfer Agreement with Dr. Carroll at Medical University of South Carolina.

The most significant finding was identification of a common cell surface marker, epidermal growth factor receptor (EGFR), expressed in 75% of the cell lines tested thus far. The secondary marker, S100B has been confirmed to be expressed in the five lines tested. Thus, the reagents to be used in the assay have been identified and their use optimized in one assay. The following step is to run simulation assays with normal blood samples to which model MPNST cells are added. We then will be ready to work with clinical samples in Aims 2 and 3. The long-term potential impact of the work remains to develop an assay capable of detecting

circulating MPNST tumor cells in at risk children with NF1 allowing for early detection of this malignant tumor.

Follow on Funding: None at the time of reporting.

Collaborations: All training has been conducted in the College of Medicine, Burnett School of Biomedical Sciences, working with pre-doctoral graduate, medical and undergraduate students at the Health Science Campus of the University of Central Florida in Orlando, FL.

Dr. Julianne Huegel is a staff member who joined the lab in October 2020. She was supported by this award. She designed and conducted bead capture experiments to confirm that the chosen cell surface marker can be used to isolate cells of interest. She is aided by Parth Patel.

Rosa Rosario is a senior technician specialized in animal husbandry and was recently trained in cell culture. She has been the primary cell culture specialist for this project.

Dr. Alejandra Petrilli was a senior scientist in the lab. Dr. Petrilli had been with the lab for 10 years prior to the award. She helped design several and execute several preliminary experiments, including MPNST literature research on receptors to consider. Dr. Petrilli also helped train several students in flow cytometry and immunostaining. She left her position in April.

Parth Patel is a medical student who joined the lab in March 2021. Parth has learned immunostaining and has conducted the majority of immunostaining experiments. Parth has provided support to Dr. Huegel during the antibody binding experiments.

Haley Hardin, MS is a pre-doctoral graduate student who joined the lab in October 2020. She has learned and conducted flow cytometry experiments for the project. Haley also supports Rosa Rosario with cell culture and Parth Patel with immunostaining.

Maria Martinelli, MS is a pre-doctoral graduate student who joined the lab in December 2020. She provided support to Parth Patel for immunostaining experiments and western blots and assists in cell culture.

Jessica Killingsworth is a graduate student who was with the lab for a six week rotation from September-October. Jessica trained on flow cytometry and experimental design.

Jenna Aquino is a graduate student who was with the lab for a six week rotation from October-November. Jenna was trained in both flow cytometry and immunostaining.

Parth Chandan is an undergraduate student of the University of Central Florida in the Burnett School of Biomedical Sciences. He has trained on western blots and imaging immunofluorescence.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Li-Mei Chen, MD, PhD

Organization: University of Central Florida

Abstract: Non-Hodgkin lymphoma (NHL) is a type of cancer originating from cells that constitute the body's immune system. Each year in the US, there are about 800 children diagnosed with NHL, which ranks as the third most common malignancy in children and accounts for 7% of all childhood cancers. Current treatments for NHL are mostly chemotherapy and surgery for early stages of the cancer. Although the five year survival rate can reach to over 80%, the prognosis is very poor in patients with recurrent disease or refractory to the first-line chemotherapy. There is no standard treatment for chemo-resistant patients and the survival rate is at 10-30%, presenting an unmet challenge.

In NHL, Burkitt lymphoma is the fastest growing and a very aggressive tumor in humans. Previous studies have shown that a cell membrane-anchored proteolytic cutting enzyme/protein, matriptase is often over-expressed in Burkitt lymphoma. Down-regulation of matriptase expression reduced tumor growth in a mouse model.

Matriptase is an enzyme, once activated, can auto-activate itself and eliminate its occupancy on the cell membrane. To reduce/remove the matriptase protein in lymphoma cancer cells, a method was developed in which biologically engineered- small membrane particles, exosomes, carrying a matriptase-activating agent, the prostasin protein, are used to activate and reduce the matriptase protein in lymphoma cancer cells. The purpose of this study is to establish a prostasin-matriptase proteolytic cutting-cascade on cell membranes and to remove matriptase from the cancerous lymphoma cells.

In the first year of this study, prostasin-exosomes were successfully constructed and characterized. The results were summarized and published in Bioscience Reports. The functionality of these particles were further evaluated. The results indicated that prostasin-exosomes can efficiently reduce the presence of matriptase in the cancerous B lymphoma cells.

In the next phase of this study, investigation of the growth rate and invasiveness of cancerous B lymphoma cells will be carried out on cells treated by prostasin-exosomes and have a reduced level of the matriptase protein. The purpose of this study is to determine if the activated prostasin-matriptase cascade on the cancerous lymphoma cells lead to a reduced tumor growth and invasiveness.

If successful, this method can be tested in a future preclinical study with a Patient-Derived Xenograft model. Activation of protease-cascades on cancer cell membranes as a means to eliminate cancer cells can be an alternative approach for treating B cell lymphoma.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of this reporting.

Journals: Li-Mei Chen, Julius C Chai, Bin Liu, Tara M Strutt, K Kai McKinstry, Karl X Chai. Prostasin regulates PD-L1 expression in human lung cancer cells. Biosci Rep. (2021) doi:

10.1042/BCJ20210407. PMID: 34195807 DOI: 10.1042/BCJ20210407. https://doi.org/10.1042/BCJ20210407 https://pubmed.ncbi.nlm.nih.gov/34195807/

Patents: None at the time of reporting.

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

Principal Investigator: Elias Sayour, MD, PhD

Organization: University of Florida

Abstract: The COVID-19 pandemic which has created substantial delay (nearly a year) in initiating this complex study. When the pandemic hit, the University (UF) shut down and put holds on equipment purchases and onboarding of new personnel. We could not purchase equipment or onboard new personnel to set up Qa/Qc workflows and manufacture product as dictated in our approved FDA-IND (BB-19304, Sayour). When UF lifted these holds (months later) and we finally onboarded personnel (September-October 2020), we could not pursue validation of our technology due to backlogs on orders for vaccine ingredients. These ingredients were not received until March 2021, but validations has since taken place without significant complication.

We have set up our manufacturing queue for this first-in-human trial with validation for quality assurance and release testing.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of this reporting.

Journals: Melnick K, Dastmalchi F, Mitchell D, Rahman M, Sayour EJ. Contemporary RNA Therapeutics for Glioblastoma (2021). Neuromolecular Med. (2021) doi:10.1007/s12017-021-08669-9

Patents: None at the time of reporting.

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

Principal Investigator: Coy D. Heldermon, MD, PhD

Organization: University of Florida

Abstract: The COVID-19 pandemic delayed start of project due to mouse colony restrictions and personnel distancing requirements however we have subsequently expanded the p53 mouse colony. Many mice have developed tumors at >5 months of age with most male mice developing a testicular tumor. We have passaged a few tumors from mice that may be sarcomas in order to facilitate the grant aims and at least one seems consistent with sarcoma by immunohistochemistry. We are confirming identity using RNA sequencing currently and if sequencing is consistent with sarcoma will complete the aims using isografts from this tumor given the consistent and shortened time to tumor development.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of this reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Julio C. Barredo, MD

Organization: University of Miami

Abstract: This grant proposes to elucidate the mechanism(s) of epigenetic control of metabolic stress responses in Acute Lymphoblastic Leukemia (ALL) cells regulated by AMP-activated kinase (AMPK) via interactions with chromatin-associated factors, and to exploit unique opportunities for translation of epigenetic-based therapies. We used chromatin immunoprecipitation sequencing (ChIP-seq) assays to confirm our ChIP-seq data which uncovered that AMPKα2 is directly associated to chromatin to regulate epigenetically gene expression in CCRF-CEM/HA-NH2-AMPKα2 (CN2) cells under metabolic stress. To correlate the level of mRNA expression with recruitment of AMPKα2 to chromatin gene loci regulated in response to metabolic stress, we used Ribonucleic acid (RNA) RNA-seq assays in CN2 cells treated ± glucose deprivation, and found that about two-thirds of messenger RNAs (mRNAs) were downregulated whereas the remaining were upregulated following AMPK activation. Among downregulated mRNAs, we uncovered a cluster of histone genes that were mainly downregulated in response to metabolic stress. We used RT-qPCR and ChIP-qPCR assays to confirm and validate our data on selected histone gene candidates (HIST1H1C, HIST1H1D, HIST1H4D) which exhibited both decreased recruitment of HA-AMPKα2 to chromatin and mRNA downregulation in response to metabolic stress. We observed similar data in CN2 cells treated with other metabolic stressors/AMPK activators (AICAR, 2DG). To investigate the role and function of AMPK (AMPKα1/AMPKα2) on the regulation of histone gene expression, we

used mouse embryonic fibroblast (MEF) cell line models with wild type AMPK (wt), AMPKα1 knockout (KO), AMPKα2KO, and AMPKα1/AMPKα2 double KO (DKO) treated ± metabolic stressors. Similar to our findings in CN2 cells, we found that metabolic stress induced histone gene mRNA downregulation in all MEF cells examined, and that AMPKα1 and AMPKα2 participate in histone gene transcription in response to energy/metabolic stress, supporting the notion that both AMPKα1 and AMPKα2 may play a role to epigenetically regulate and reprogram gene expression in response to energy/metabolic stress. To further confirm our ChIP-seq and ChIP-qPCR data in MEF cells, we generated MEF monoclonal stable cell lines expressing HA-AMPKα1 or HA-AMPKα2. Using genetic constructs encoding constitutively active forms of AMPKα2, we found that expression of constitutively active AMPKα2(T172D) in CCRF-CEM and MEF DKO cells led to decrease expression of selected histone genes, indicating AMPK kinase activity was responsible for histone gene downregulation. Using published data and immunoblots, we analyzed the levels of mRNA and protein expression of each of the AMPK subunits, and found that PRKAA1/AMPKα1, PRKAB1/AMPKβ1, and PRKAG1/AMPKγ1 were the most abundant mRNA/protein subunits to be expressed in ALL cells. In contrast, PRKAA2/AMPKα2 mRNA/protein expression was detected in selected ALL cells (KASUMI-2, KE-37, JURKAT, PF-382). These data suggest that AMPKβ1 and AMPKγ1 may also interact with members of the putative AMPK/chromatin-associated complex. Using shRNA, we investigated the molecular interactions between AMPKα2 and TAF1, a putative member of the AMPK/chromatin-associated complex, and found that downregulation of TAF1 led to significant decreased of histone mRNA expression as compared to control. Using ChIP-qPCR, we determined/confirmed that recruitment of TAF1 on the chromatin of histone gene loci was decreased following AMPK activation, indicating that both AMPKα2 and TAF1 expression are required for optimal histone gene transcription in ALL in response to energy/metabolic stress.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of this reporting.

Journals: None at the time of this reporting.

Patents: None at the time of reporting.

Live Like Bella Pediatric Cancer Research Initiative
Appendix X
Fiscal Year 2020-2021 Active Grants
Funding Fiscal Year 2018-2029

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
9LA01	Florida State University	Q.X. Amy Sang, PhD	$219,138	5/31/2023	No	Yes	No
9LA02	H. Lee Moffitt Cancer Center	Mihaela Druta, MD	$787,272	4/30/2024	No	No	No
9LA03	H. Lee Moffitt Cancer Center	Keiran Smalley, PhD	$219,138	4/30/2023	No	Yes	No
9LA04	University of Florida	Lamba Jatinder, PhD	$219,138	4/30/2023	No	No	No
9LA05	University of Florida	Zhijian Qian, PhD	$218,572	5/31/2023	No	No	No
9LA06	University of Miami	Claudia Rodrigues, PhD	$109,569	5/31/2023	No	Yes	No
9LA07	University of Miami	Anthony Capobianco, PhD	$788,89	5/31/2023	No	No	No
9LA08	University of Miami	Alan Pollack, M.D, PhD	$219,318	5/31/2023	No	Yes	No
9LA09	University of Miami	Julio C. Barredo, MD	$219,138	5/31/2022	No	No	No
9LA10	University of South Florida	Mildred Acevedo-Duncan, PhD	$771,341	7/31/2022	No	No	No

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

   Principal Investigator: Q.X. Amy Sang, PhD

   Organization: Florida State University

   Abstract: Human brain and other central nervous system cancers are common types of cancer in children. Atypical teratoid rhabdoid tumor (ATRT) is a rare and very aggressive type of human pediatric brain cancer that mostly arises from the cerebellum located at the hindbrain region. A human cerebellum brain organoid model has been built by our team using induced pluripotent stem cell (iPSC) lines [Hua et al., 2021]. This proposed project is building a novel 3-dimensional spheroid model that mimics human pediatric brain rhabdoid tumor formation. The state-of-art CRISPR-Cas9 gene editing and stem cell technologies are utilized to generate this novel human pediatric brain cancer model for future drug evaluation and development for the effective treatment of pediatric brain cancer patients. The central hypotheses are that human pediatric brain malignant rhabdoid tumor is originated from early neural progenitor cells (NPCs) after the inactivation of the SMARCB1 tumor suppressor; thus, deleting the SMARCB1 gene in early NPCs may generate a rhabdoid tumor model for therapeutic evaluation. ATRT is characterized by the biallelic inactivation of a tumor suppressor gene SMARCB1 and has a high embryonic gene expression profile. The guide RNA molecules have been designed, and CRISPR-Cas9 gene-editing technology has been used to knock out the SMARCB1 gene to mimic human ATRT development in childhood. The gene knockout construct was transfected into induced pluripotent stem cells and experiments are performed to verify if the SMARCB1 gene is knocked out. DNA sequencing experiments will be carried out to verify the gene knockout, and Western

blot experiments will further verify that the SMARCB1 protein is not produced by the stem cells or the neural spheres. For this reporting period, cell lines with more than 30% of mutated SMARCB1 expression were characterized for their morphology. They are also being differentiated into spheroids using neural progenitor protocol. In addition, one of the clones was used for further SMARCB1 transfections using the same combination of SMARCB1 and Tp53 targeted CRISPR-Cas9 plasmid. The transfected cells that expressed the markers of interest were collected and replated as single cells. Western blot analysis for these new cell lines showed a low SMARCB1 expression and diminished endogenous control proteins. By combining the knockout of SMARCB1 with Tp53 could lead to cancerous development. We have completed Specific Aim 1, Task 1, and Task 2 and have made very good progress with the most challenging Task 3. Recently, we have generated new SMARCB1 knockout clones and are able to knockdown the SMARCB1 protein expression by ~80%, according to the previous Western blot results. We are in the process of characterizing these clones as well as obtain more clones with complete SMARCB1 knockout and examining the morphology of these mutated clones and ATRT cell lines

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of this reporting.

Journals: Cerebellar Differentiation from Human Stem Cells Through Retinoid, Wnt, and Sonic Hedgehog Pathways. Hua TT, Bejoy J, Song L, Wang Z, Zeng Z, Zhou Y, Li Y, Sang QA. Tissue Eng Part A. (2021) doi: 10.1089/ten.TEA.2020.0135. Epub 2020 Oct 1. PMID: 32873223

Patents: None at the time of this reporting.

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

Principal Investigator: Mihaela Druta, MD

Organization: H. Lee Moffitt Cancer Center

Abstract: The purpose of the study is to see if Nivolumab (Dose Level 1) or Nivolumab in combination with Azacitidine (Dose Levels 2 and 3) given to patients before and after surgery is safe and to see if patients are able to successfully complete the treatment before their surgery without any extended delays in treatment. As of June 30, 2021, the research team has had a total of 12 patients (1 had to be replaced due to withdrawal prior to treatment) accrued for this study. The first 6 patients were accrued on Dose Level 1 where no dose limiting toxicities (DLTs) were reported during the DLT time period, no serious adverse events were caused by being on therapy, and there were no delays for surgery. Prior to proceeding to Dose Level 2, an interim analysis of Dose Level 1 was submitted to the Protocol Monitoring Committee for review and was approved end of July 2020. Dose level 2 has a 3+3 study design which means that based on how the first three patients perform on the trial will determine whether three more should be added at this same dose level. We enrolled the first three patients, however, the third patient on Dose level 2 experienced a dose limiting toxicity (side effect) on this dose level so 3

additional patients will be added to Dose Level 2. Currently two out of the three slots have been filled with neither patient experience any DLTs. We are currently waiting to fill the last slot. All patients are off treatment due to disease progression or withdrawal. A total of nineteen sites have been activated and are open to enrollment (five sites in Florida and fourteen sites outside of Florida - an additional 4 sites are pending activation).During our last quarterly meeting with our Clinical Trials Oversight Committee on July 21, 2021, it was decided for the study to continue as designed (doctors not related to the Sunshine Trials review our trials and let us know if we can proceed or if there any red flags).

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of this reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Keiran Smalley, PhD

Organization: H. Lee Moffitt Cancer Center

Abstract: Melanoma is the deadliest form of skin cancer. Although most commonly considered to be an adult disease, melanoma can also occur in pediatric patients. The incidence of pediatric melanoma is rising, particularly in individuals between the ages of 15-19. Moffitt Cancer Center has developed an integrated program for pediatric melanoma management with one of the largest patient populations in the world. The majority of pediatric melanomas are sporadic and relatively little is known about their molecular basis or the precise etiologic mechanisms. Although there is evidence that neonates and young children have less developed immune systems and may also be uniquely susceptible to the mutagenic effects of ultraviolet radiation (UVR), the interplay of these two risk factors in melanoma development is not known. In the second year of the grant we have studied the differences in immune responses to UVR irradiation in young and old mice, and then the influence of UVR upon subsequent melanoma development. We have made significant progress in understanding the age-related nature of the immune response to UVR. It was found that younger mice had a much impaired immune response when UV-irradiated compared to the older mice, this was observed in both the lymphocyte and myeloid cell compartments. Samples have been collected from UVR treated mouse skin and processed for single cell RNA-Seq (scRNA-Seq) analysis. These data have been subjected to quality control and are now being analyzed and validated by immunohistochemistry. In preparation for these analyses we have developed several important new bioinformatic tools and methods to curate mouse immune cell populations. This has already resulted in one publication in the journal Cancer Immunology Research. We additionally developed new methods to map complex cell-cell intercations in the mouse melanoma immune environment. In our second series of studies we have followed cohorts of mice exposed to UVR at both neonatal and adult ages, in whom a potent oncogene (BRAF V600E) was induced at day 22, for the development of melanomas. Both the pediatric and adult groups developed

melanomas, and unexpectedly the mice receiving radiation at the neonatal stage showed a longer tumor-free survival than mice irradiated as adults. Tumors have been harvested and processed for histopathology, multiplexed immunohistochemistry for immune cell subsets, and scRNAseq, and the peripheral blood was analyzed by flow cytometry. An analysis of the tumor immune infiltrate from the mice irradiated as neonates and adults is ongoing. Early signs suggest that T cells infiltrating the adult tumors are lower in effector molecules such as Granzyme B and that the tumor associated macrophages from adult tumors are higher in expression of immune checkpoints such as PD-L1. Further analyses are ongoing to determine why initially poor immune responses to UVR in the pediatric mice later translate into better anti-tumor immunity. In year three of this work we expect to determine the effects of age of sun exposure upon melanoma development, allowing new prevention and treatment strategies to be developed.

Follow on Funding: None at the time of reporting.

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

Journals: Phadke MS, Chen Z, Li J, Mohamed E, Davies MA, Smalley I, Duckett DR, Palve V, Czerniecki BJ, Forsyth PA, Noyes D, Adeegbe DO, Eroglu Z, Nguyen KT, Tsai KY, Rix U, Burd CE, Chen YA, Rodriguez PC, Smalley KSM. Targeted Therapy Given after Anti-PD-1 Leads to Prolonged Responses in Mouse Melanoma Models through Sustained Antitumor Immunity. Cancer Immunol Res. (2021) doi: 10.1158/2326-6066.CIR-20-0905. Epub 2021 Mar 2. PMID: 33653716; PMCID: PMC8102376.

Patents: None at this time of reporting.

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

Principal Investigator: Lamba Jatinder, PhD

Organization: University of Florida

Abstract: The three main investigators have held regular in person and video-conference monthly meetings in order to discuss implementation of the experimental plan, data acquisition and patient recruitment. Since last report on the analysis results on the first batch of patients we have acquired 42 additional specimens (making the total number to be 92+42= 132). i) drug levels are being quantitated in the new batch which will be followed by PK analysis. ii) Genotyping for 105 SNPs in the second batch of samples has been completed and QCed. Once the PK and clinical outcomes endpoints are completed, we will begin statistical analysis association between SNPs and phenotypic endpoints. In the mean time we have completed the data analysis of batch one and have it prepared an abstract for submission to American Society of Hemtology (ASH) for presentation the upcoming annual meeting to be held in Atlanta from Dec 11-14th, 2021.

Total body irradiation (TBI) conditioning regimens are considered standard of care for pediatric hematopoietic cell transplant patients (HSCT). TBI is known to cause late toxicity, so non-TBI regimens have been used successfully. This led to a recent multi-institution prospective clinical trial using a non-TBI multidrug conditioning regimen that included busulfan, fludarabine, and/or thiotepa (BuFluTT). This study's goal was to investigate BuFluTT's pharmacogenomic effects on HCT outcomes such as drug-related PK profiles, disease relapse and re-transplantation.

This ongoing study included pediatric patients aged one to 25 years from the PBMTC nationwide study receiving BuFluTT as part of pre-transplant conditioning. Genomic DNA was obtained from peripheral blood. SNPs in candidate pharmacological genes in BuFluTT pathways with a minimum allele frequency of 10% were chosen from literature searches and PharmGKB databases. Using logistic regression models from SNPassoc R package, SNPs were tested for association with individual drug PK and 1-month post-transplant chimerism with CD3+ (sufficient as donor >80%) and CD14/15+ (sufficient as donor >95%) across all mode of inheritance: codominant (A/A vs. A/B vs. B/B), dominant (A/B-B/B vs A/A), recessive (B/B vs. A/A-A/B), over-dominant (A/B vs. A/A-B/B), and log-additive (A/A=0, A/B=1, B/B=2) modes of inheritance, where A is reference and B is alternative alleles. Gray's methods were used to estimate and compare the cumulative incidence of clinical outcomes associated with disease relapse and re-transplantation 3-years after the 1st transplantation, while controlling for competing risks for each SNP in each mode of inheritance. SNPs with an association p-value <0.05 were considered significant. When multiple modes of inheritance are significant per SNP, the mode of inheritance determined by the lowest p-value and/or Akaike information criterion (AIC).

In total, 87 patients included in the preliminary analysis had a median age of three and one-half (0.2 - 17.9) years, were 63% male, 48% Caucasian, 85% receiving allogenic hematopoietic cell transplantation, and 43% had malignant hematologic disease. Among patients with available cumulative area-under-the-curve (cAUC) info, the median cAUC for Busulfan was 68 mg•hr/L, Fludarabine was 3.72 mg•hr/L, and Thiotepa was 13.9 mg•hr/L (Table 1). After excluding 8 SNPs that deviated from Hardy–Weinberg equilibrium, 62 SNPs were used for downstream association tests, yielding 30 significant SNPs (Table 2). Significant difference in cAUC of busulfan is predicted by the following relevant SNPs: rs4715354_GSTA5 has A/G with lower level than A/A-G/G by 12.3 (3.1-21.6, over-dominant), rs11577910_CTPS1 has G/A with lower level than G/G-A/A by 14.6 (3.3-25.7, over-dominant), rs12144160_CTPS1 has increased level with 8.6 from G/G to G/A to A/A (2-15.2, log-additive), and rs7254579_CYP2B6 has decreased level with 7.2 from T/T to C/T to C/C (1-13.4, log-additive). Significant difference in cAUC of fludarabine is predicted by the following relevant SNP: rs3754446_GSTM5 has C/A with higher level than A/A-C/C by 0.56 (0.12-1, over-dominant); and for changes in cAUC for thiotepa: rs1057910_CYP2C9 has C/A-C/C with higher level than A/A by 11.3 (2.2, 20.4, dominant). In codominant, dominant, and log-additive inheritance modes, rs12144160_CTPS1 has zero odd of being sufficient when comparing level of donor chimerism in both CD3+ and CD14/15+ at 1-month. Regarding disease relapse and retransplant with alternative allele associated with worst endpoints, rs2277119_CYP39A1 has T/C with HR of 4.45 (1.4-14.5, over-dominant) compared to C/C-T/T, and rs2279343_CYP2B6 has G/G with HR of 6.51 (1.54-27.43, recessive) compared to AA-A/G (Figure 1). Besides predicting busulfan cAUC level, rs4715354_GSTA5 is

also associated with better outcome in disease relapse and retransplant as A/G-G/G has HR of 0.32 (0.11-0.95, dominant) compared to A/A (Figure 2).

We have identified SNPs in the pharmacology genes predicted interpatient variability in BuFluTT PK drug profiles and one-month donor chimerism. While two SNPs in CYP39A1 and CYP2B6 have the worst outcome in disease relapse and retransplant after three years, one SNP in GSTA5 has a better clinical outcome and predictive of busulfan cAUC level.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of this reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Zhijian Qian, PhD

Organization: University of Florida

Abstract: This study aims to get a better understanding of the biology of a subset of Pediatric Acute Myeloid Leukemia (AML), which is one of the most common and fatal forms of hematologic malignancies. Ecotropic virus integration stie-1 (EVI1) high expression was detected in 10-25% of pediatric and young adult AML with an adverse outcome in these patients. No targeted or individualized therapies on this subset of AML patients are available.

During this period of time, we have made significantly progress on this project. Our study focused on determining how EVI1 high expression contributes to the development of MDS/AML using our newly established animal model, in which the EVI1 gene can be induced to mimic the upregulation of EVI1 gene expression in hematopoietic stem/progenitor cells (HSPCs) from MDS/AML patients with high EVI1 expression. To understand the molecular mechanisms underlying the role of EVI1 in the development of myeloid malignant diseases, we have performed global gene expression HSPCs from both the control and EVI1 transgenic mice. We have identified several interesting downstream targets of EVI1. We further validated the expression of these candidate genes by quantitative qRT-PCR in the HSPCs from these mice. Among these candidate genes, Neurogenin 1 (Ngn1) gene stands out as its unique function in regulation of neuronal differentiation through induction of microRNA 9 (miR9). The function of Ngn1 in normal hematopoiesis and leukemogenesis is unknown. Of interest, we found that NGN1 gene overexpression significantly promoted cell survival and proliferation. We further demonstrated that Ngn1 knockdown reversed EVI1-induced abnormal cell growth of HSPCs in vitro. These results suggest that Ngn1 is a critical downstream target of EVI1, and its function may be also mediated at least partially by miR9 in HSPCs.

Our study provides a new insight into getting into the molecular mechanism that mediates the function of EVI1 in leukemogenesis, which facilitates the identification of new therapeutic strategies for the treatment of children patients with AML.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of this reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Claudia Rodrigues, PhD

Organization: University of Miami

Abstract: The Rodrigues laboratory at the University of Miami is investigating mechanisms underlying the toxic effects of chemotherapy agents to the heart. Cancer survivors, especially children, experience serious chronic and life-threatening effects that can lead to cardiovascular disease and heart failure. Currently there is no effective treatment against chemotherapy toxicity. The goal of the present studies is to identify early and late mechanisms involved in doxorubicin toxicity that can be targeted for the development of novel cardioprotective therapies.

During the past two years, Dr. Rodrigues laboratory has been investigating the role of a molecule known as c-Myc in cardioprotection after exposure to one of the most common chemotherapy agents used in cancer treatment, doxorubicin. Dr. Rodrigues and her research group have found that lowering the levels of c-Myc in cells that line our blood vessels, minimize acute injury to the heart and attenuate cardiovascular dysfunction. In the past year, Dr. Rodrigues lab has performed analysis of molecular messengers in the heart of animals lacking c-Myc in blood vessels and found that one of the potential mechanisms by which this molecule protects the heart from chemotherapy-induced injury may be through regulation of the circadian rhythm. Dr. Rodrigues' group findings have just been accepted for an oral poster presentation at the American Heart Association Basic Cardiovascular Sciences Scientific Sessions 2021. Furthermore, based on findings originated from this current Live Like Bella pilot award, Dr. Rodrigues has been able to secure a new grant from the Florida Heart Research Institute to further investigate how the circardiam rhythm can be targeted as cardioprotective strategy during chemotherapy exposure. This is an expansion of the current work.

Dr. Rodrigues group has completed a one-year longitudinal study in the current period of this award and found significant results indicating that the toxic effects of doxorubicin are sex-specific, suggesting that males and females require different cardioprotective treatment. In addition, these studies have shown that the heart of animals exposed to doxorubicin fail to undergo age-associated adaptive changes, which could lead to cardiac dysfunction. In the next period, last year of this pilot award, Dr. Rodrigues lab will complete the molecular analysis of control and chemotherapy-exposed heart samples collected from acute and aged experimental models. Analysis of this data is expected to lead to novel information that can be used in future studies to prevent cardiotoxicity of chemotherapy agents or related cardiomyopathy.

Follow on Funding: FHRI, Molecular Mechanisms of Anthracycline-Induced Cardiovascular Toxicity. Claudia Rodrigues, $156,400.

Collaborations: None at the time of this reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Anthony Capobianco, PhD

Organization: University of Miami

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

Aim 1: Identify novel regulators of GLI2 required for TRP53 mutant SHH medulloblastoma viability. This year we primarily investigated the role the DNA methyltransferase DNMT1 plays as a novel Gli regulator in MB progression. We focused on DNMT1 because it was a validated hit in our Gli regulator screen, higher DNMT1 expression is associated with a worse prognosis in a retrospective cohort of MB patients, and clinically relevant inhibitors are available that could be repurposed for MB patients. This year we showed that DNMT1 is required in SHH agonist treated iMEFs and SUFU null iMEFs, which exhibit constitutive SHH activity, for maximal Gli activity. This result places DNMT1 downstream of primary cilia in its regulation of Gli activity.

We showed that DNMT1 knockdown attenuates the viability of SHH-subgroup MB sphere cultures and that it does so in a manner that reduces the expression of Gli biomarkers. We were also able to show that the DNMT1 inhibitor, 5-Azacytidine, is able to reduce MB cell viability and Gli biomarkers in similar MB sphere cultures. Preliminary data using 5-Azacytidine in a mouse model of MB showed that this DNMT1 inhibitor reduced tumor growth and proliferation markers. We are in the process of showing that 5-Azacytidine attenuates SHH-subgroup MB growth in an on-target manner.

We have begun to elucidate the relationship between DNMT1 and Gli proteins. We have shown that DNMT1 co-immunoprecipitated with Gli2 from MB cell cultures. Further, we were able to show that Gli2 and DNMT1 are part of a large macromolecular complex, along with both HDAC1 and HDAC2. We are in the process of screening through other potential members of this complex, using other hits identified in our siRNA screen. We were also able to enrich this protein complex on Gli-binding site oligonucleotide beads, but not control oligonucleotide beads. Efforts to localize this macromolecular Gli complex to Gli target genes using ChIP analysis is underway.

We have also made progress in organizing and writing a manuscript draft highlighting some of this work. As part of these efforts we analyzed various MB single cell RNA seq data, our own data and published data, and identified co-localization of various members of the Gli complex described above in distinct cell populations. We then began to examine the grouping of various other hits from our previously described siRNA screen in these distinct cell populations. These efforts are also on going.

Aim 2: Identify regulators of tumor propagating cell viability in TRP53 mutant medulloblastoma. Our preliminary data suggest that the ability of the SOX2+ MB cells to self-renew is regulated by the small non-coding RNA miR34a. In these cells, loss of P53 reduces the expression of miR34a, triggering the activation of a series of miR34a-repressed signaling networks that control tumor propagation, including WNT signaling. We carried out a candidate approach centered on miR34a-repressed targets and an approach focused on identifying a stemness enriched cell cluster in SHH MB tissues using single cell RNA sequencing analyses. Candidate pathways identified using both approaches were then prioritized based on MB patient outcomes1. Our data allowed us to identify the EPH/EPHRIN signaling pathway as a novel candidate regulator of TRP53 mutant MB self-renewal- which we are in the process of writing up.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of this reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Alan Pollack, MD, PhD

Organization: University of Miami

Abstract: The objective is to investigate the mechanistic role of Sphingomyelin Phosphodiesterase Acid Like3B (SMPDL3b) in renal injury after a single dose and fractionated total body irradiation (TBI). Our long-term goal is to discover a molecular-based protective or mitigating strategy for radiation nephrotoxicity (RN). We shall determine how TBI with and without Hematopoietic Stem Cell Transplantation (TBI±HSCT) affects podocytopathy and RN. We will use SMPDL3b wild type (SMP-WT), SMPDL3b knockout (SMP-KO), and SMPDL3b

overexpressing (SMP-OE) human podocyte and podocyte- specific SMP-KO mice to investigate the role of SMPDL3b in RN. Then, we will determine if targeting SMP with rituximab mitigates TBI-induced renal injury.

As proposed in Aim1, we have established that glomerular surface area decreases, Tumor Necrosis Factor-alpha (TNF- α) expression increases 10 weeks after "Focal" bilateral kidney irradiation (RT). However, we did not observe a significant change in TNF- α expression over the time post-RT. We have established that Arginyltransferase 1 (ATE1), SMPDL3b expression, podocyte number, and kidney function decrease in a dose and time-dependent manner after RT. However, fractionated equivalent does (FRT) partially reduced the risk of kidney injury at 20 weeks post-RT. We have established that TBI±HSCT causes more renal damage than focal RT. Treatment of bone marrow (BM) with CD4+ plus CD8+ T-cell further enhances the severity of RN.

As proposed in Aim 1., we have established that SMP-KO mice are more susceptible to radiation-induced DNA damage and apoptosis than SMP-WT mice. We have established that rituximab antibody pretreatment preserves the expression of SMPDL3b and ATE1 and prevents podocytopathy, and improves renal function.

As proposed in Aim 2, we have established that RT changes the sphingolipid homeostasis in human glomerular endothelial cells (hGEC) and podocytes. We have established that total ceramide and ceramide-1-phosphate (C1P) levels increase, and sphingosine-1-phosphate (S1P) decreases after RT. We have shown that increased levels of C1P cause podocyte apoptosis. We have established that the levels of ceramide, C1P, and S1P do not change by irradiation in SMP-OE human podocytes. We have established that SMPDL3b overexpression prevents radiation-induced podocyte damage and improves podocyte survival.

The proposed research significantly impacts Floridians' health because it describes SMPDL3b as a novel target of radiation- induced renal injury. It offers the possibility that targeting SMPDL3b may represent a new therapeutic approach to prevent radiation-induced renal injury that can occur after cancer therapy. Therefore, this project will help to improve the health of Floridians living with cancer or long-term survivors, especially those treated with oncologic radiotherapy involving abdominal-based tumors. In addition, the clinical care of patients requiring treatment with TBI would also improve. Low- dose TBI is an effective conditioning regimen for hematopoietic stem cell transplantation. However, this is associated with acute and chronic adverse normal tissue toxicities. Our proposal directly addresses one aspect of reducing renal toxicity. The overall goal of our research is to develop a novel therapy and strategy to mitigate the effects of radiation on RN. This project will benefit Floridians by reducing mortality and morbidity, resulting in a significant health benefit for this patient population.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of this reporting.

Journals: Ahmad A, Shi J, Ansari S, Afaghani J, Molina J, Pollack A, Merscher S, Zeidan YH, Fornoni A, Marples B. Non-invasive Assessment of Radiation-Induced Renal Injury in Mice. Int J Radiat Biol. (2021) DOI: 10.1080/09553002.2021.1876950. PMID: 33464992.

Matthew B. Wright, Javier Varona Santos, Christian Kemmer, Cyrille Maugeais1, Jean-Philippe Carralot, Stephan Roever, Judith Molina, Anis Ahmad, G. Michelle Ducasa, Alla Mitrofanova, Alexis Sloan, Christopher Pedigo, Mengyuan Ge, Jeffrey Pressley, Laura Barisoni, Armando Mendez, Jacopo Sgrignani, Andrea Cavalli, Sandra Merscher, Marco Prunotto, Alessia Fornoni. Novel small molecule compounds targeting OSBPL7 increase ABCA1-dependent cholesterol efflux and preserve renal function in mouse models of FSGS and Alport Syndrome. Nature Communications. (2021) (Accepted; referencenumber: NCOMMS-20-43282).

Marina Francis, Larry Bodgi, Anis Ahmad, Patrick Azzam, Tarek Youssef, Alaa Abou Daher M.S1, Assaad, A. Eid DSc1, Alessia Fornoni, Alan Pollack, MD, Brian Marples, and Youssef H. Zeidan. SMPDL3b Modulates Radiation-Induced DNA Damage Response in Renal Podocytes. FASEB. (2021) (Under review; Manuscript # 202100186).

Patents: None at the time of reporting.

9. **Grant #:** 9LA09 Enhancing Immunotherapy Through Inhibition of Carbonic Anhydrase IX to Treat Osteosarcoma

Principal Investigator: Julio C. Barredo, MD

Organization: University of Miami

Abstract: This grant proposes to evaluate whether WBI-5111 (a carbonic anhydrase inhibitor) can augment anti-PD1 therapy in primary and metastatic osteosarcoma and to investigate the molecular mechanisms involved. We used the intraosseous model we proposed in our application based on implantation of K7M2-luciferase labeled cells in the tibia of BalbC mice. We showed successful implantation of these cells in our intraosseous model and preliminarily in vivo treatment with the Carbonic Anhydrase IX (CAIX) inhibitor WBI-5111 plus an anti-PD-1 Ab led to decrease in tumor burden compared to control and single agent treatment (detected using bioluminescence imaging). More in depth in vitro testing of available CA9 inhibitors showed that while CA9 is expressed under hypoxia and hypoxia mimetics like cobalt chloride (CoCl2), there was no significant change in K7M2 cells' viability. This result differs from our preliminary data/experience generated in other cancer models such as pancreatic cancer (refer to original application). Since the effect of CA9 inhibition with anti-PD1 treatment in our hands showed modest effect on overall survival (OS), we moved to test Lactate Dehydrogenase (LDH) inhibitors (LDHi) (to mimic CA9 inhibition). Concentrated efforts in optimizing the humanized model to test this combination in primary human OS cells. We implanted patient derived tumor cells on the right tibia of NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice and monitored engraftment. Lack of weight loss indicated absence of GvHD. To determine if the patient-derived xenografts transplanted in the tibia of NSG mice had engrafted, we took advantage of the ability of osteosarcomas to deregulate bone remodeling. In this preliminary computed tomography (CT) study, analysis was focused on the diaphysis of the tibia (tibial midshaft). CT attenuation analysis of the tibia-cortical region, measured in Hounsfield units (HU), was then used to estimate potential changes in bone density. While no extensive changes were observed, a noticeable increase in the density of the bone of the right tibiae was detected. This analysis showed that the volume occupied by bone tissue in the right tibiae of the four mice

tested is bigger than in the control left tibiae, suggesting a trend in bone remodeling after tumor transplantation. HE analysis also revealed no metastasis development in the lungs. Since there was no tumor tissue available to evaluate human immune cell infiltration, we focused on understanding human immune engraftment on spleen samples (to allow further develop the humanized model). Markers of activation and immune suppression were analyzed in the lymphoid/myeloid compartment using spectral flow cytometry. Mice from CTRL, LDHi, and LDHi+PD-1 groups had~80% of human CD45+cells in the spleen. However, the anti-PD-1 group showed 40% of human cell engraftment. in all groups, human progenitor cells were able to differentiate in T cells, B cells, and NK cells. However, monocytes and neutrophils were not abundant. Since we have been able to show humanization but lack of engraftment by primary OS cells, ongoing efforts focus on overcoming the absence of engraftment by further increasing humanization and/or optimizing implantation (injection of higher number of cells and/or optimizing timing of injection), among other strategies.

These mice were treated with LDHi plus anti-PD-1 Ab and at day 40 the legs and lungs of sacrificed mice were processed for histopathology evaluation.

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 repoting.

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

Principal Investigator: Mildred Acevedo-Duncan, PhD

Organization: University of South Florida

Abstract: We have tested two atypical protein kinase inhibitors as therapeutic candidates against neuroblastoma cells. 5-amino-1-2,3-dihydroxy-4-(methylcyclopentyl)-1H-imidazole-4-carboxamide (ICA-1S), is a PKC-ι specific inhibitor and 8-hydroxy-1,3,6-naphthalenetrisulfonic acid (ζ-Stat) is a PKC- ζ specific inhibitor. Previously in past reports we have shown that the inhibitors lead to decrease cell proliferation across our various cell lines, as well as showing initial molecular pathway effects. In addition, we have reported the effects of ICA-1S and ζ-Stat on the molecular pathways that regulation cell cycle progression and metastasis in relation to aPKC/ CDK2/7/NFκB and Vimentin/ aPKC pathways. Our results demonstrated that while these drugs downregulate key markers in these pathways. To achieve greater apoptosis, the changes are not uniformed seen across the different cell lines. This is further backed by the disease state already being associated with a high degree of variation across patient types.

Our preliminary data indicates that 14-3-3 acts as a central key regulator in of aPKC driven cell cycle progression in BE-2C and BE-M17 cells. In addition, we found that Smad2/3 plays a vital role in upregulation of aPKC driven Vimentin dynamics in these cell lines in relation to 14-3-3 and aPKCs.

The 14-3-3 proteins are a family of conserved regulatory molecules that are expressed in all eukaryotic cells. 14-3-3 proteins have the ability to bind a multitude of functionally diverse signaling proteins, including kinases, phosphatases, and transmembrane receptors. More than 200 signaling proteins have been reported as 14-3-3 ligands. 14-3-3 is known to bind PDK1, BAD, Akt1 and c-Raf and perform various tasks. 14-3-3 proteins also contribute to the auto-inhibition. As 14-3-3 proteins are all known to form constitutive dimers, their assemblies have two binding sites. Thus, the dimer acts as a "molecular handcuff", locking their binding partners at a fixed distance and orientation. When the precisely positioned twin 14-3-3 binding motifs are engaged by a single 14-3-3 protein dimer, they become locked into a conformation that promotes auto-inhibition and does not allow the disengagement of the autoinhibitory and catalytic domains. Unphosphorylated 14-3-3 associating motifs do not bind their partners: they need to get phosphorylated on conserved serines (Ser 259 and Ser 621) first, by other protein kinases. The most important kinase implicated in this event is TGF-beta activated kinase 1 (TAK1), and the enzymes dedicated for removal of these phosphates are the protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A) complexes. Defects in Smad signaling can result in TGF-B resistance, causing dysregulation of cell growth. Deregulation of TGF-B signaling has been implicated in many cancer types, including pancreatic, colon, breast, lung and prostate cancer. TGF-b activated SMAD2/3 signaling promotes cancer progression via 14-3-3. This process is blocked as a result of aPKC KD which leads to reduced levels of SMAD2/3. In our final phase we have analyzed the effects of aPKC inhibition using specific inhibitors on the expression of 14-3-3 and SMAD2/3.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of this reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Live Like Bella Pediatric Cancer Research Initiative
Appendix Y
Fiscal Year 2020-2021 Active Grants
Funding Fiscal Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
8LA02	University of Central Florida	Cristina Fernandez-Valle, PhD	$200,000	4/30/2021	No	No	No
8LA04	Miami Cancer Institute, Baptist Health South Florida	Matthew D. Hall, MD, MBA	$700,000	4/30/2022	No	No	Yes
8LA05	Florida International University	Diana Azzam, PhD	$700,000	4/30/2022	No	Yes	No

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

   Principal Investigator: Cristina Fernandez-Valle, PhD

   Organization: University of Central Florida

   Abstract: Neurofibromatosis causes tumors to grow on nerves throughout the body (schwannomas) and in the brain (meningiomas and ependymomas). Neurofibromatosis type 2 (NF2) affects around 1 in 25,000 individuals worldwide. NF2 is caused by mutations in the Neurofibromatosis Type 2 gene (NF2) that encodes a tumor suppressor called merlin. A diagnostic criteria for this disorder is the development of schwannomas on both hearing and balance nerves; these tumors are called vestibular schwannomas or acoustic neuromas. NF2 patients develop multiple schwannomas on other nerves, and multiple brain and spinal tumors such as meningiomas and ependymomas. Most patients develop symptoms in their teenage years or during early childhood. Tumor control early in life is critical for maintaining a quality of life and preventing malignancies. Currently, potential treatments available for NF2-associated tumors are surgery, chemotherapy, and radiation therapy. However, due to location some tumors are inoperable and there are no Food and Drug Administration (FDA) approved drug therapies that shrink or stop the growth of schwannoma tumors. Undoubtedly, there is a great need for pharmaceuticals to prevent tumors from growing and shrinking or slowing the growth of existent tumors. The development of NF2 drug therapies has been challenged by lack of relevant merlin-deficient Schwann cell lines, animal models and clear druggable target because merlin lacks enzymatic activity. To address these deficits, our lab has created a panel of human and mouse cell models of the disease, developed high-throughput viability and high-content multi-parametric assays, conducted multiple screening campaigns of compound libraries using our Schwann cell lines, and optimized a sciatic nerve allograft mouse model in immune-deficient mice. We performed an unbiased chemical genomics approach and identified several PI3K and PI3K/mTOR inhibitors that selectively reduce viability NF2 model cells compared to control cells. However, kinome analysis of NF2 model cells chronically exposed to PI3K inhibitors revealed that cells compensated by consistently increasing activity of focal adhesion kinase (FAK) and

SRC kinase family members. Rewiring of kinase networks occurs in cancer cells developing drug resistance to monotherapies. Moreover, an exploratory combination drug screen identified the PI3K inhibitor GSK-2126458 and the FAK inhibitor, TAE226, as highly synergistic and selective for merlin deficient Schwann cells. These results suggest that targeting the FAK/SRC pathway in combination with PI3K inhibitors should provide sustained inhibition of merlin-deficient Schwann cell proliferation and/or survival. To advance these findings, we propose to screen the effectiveness of PI3K pathway and FAK/SRC inhibition (alone and in combination) in multiple mouse and human merlin-deficient Schwann cell lines, and to study in vivo the best synergic combination (individual and combined drugs), using our orthotopic allograft model of NF2 schwannomas. We expect to obtain the necessary preclinical data to support their potential use in NF2.To optimize translation into clinical trials, the in vivo preclinical study will be designed and conducted in consulatation with our collaborators, Drs. Smith and Aguilar-Bonilla, the pediatric neuro-oncologists at the Arnold Palmer Hospital for Children in Orlando. During the NCE, we plan to continue work on Aim 3 that entails pharmacodynamic and phenotypic studies of human and mouse model schwannoma cells treated with pictilisib and PF-03758309. We will interrogate inhibitor effects on cell cycle progression and cell death pathways in both human and mouse schwannoma model cell lines. In addition, we will conduct western blots to confirm drug target modulation and phenotypic results. Our initial apoptosis assay in human schwannoma model cell line (HS01) suggests a cell cycle arrest and no caspase 3/7 dependent apoptosis. In contrast, caspase 3/7 dependent apoptosis is observed in the mouse schwannoma model cell line treated with1.5 uM pictilisib as well as the combination in a concentration dependent manner. Subsequent studies will be conducted in both cell lines but likely only with pictilisib. We will conduct a flow cytometry EdU assay to assess cell cycle progression to complete subaim 3b. If time and resources allow we will perform RT-PCR arrays (Qiagen) to complete Aim 3a. The assays will be run in 96- or 384-well plates to assay expression level of apoptotic and/or cell cycle genes. For example, the Cell Cycle Array RT2Profiler measures expression of approximately 80 genes involved in cell cycle progression. Similar arrays are available for cell death pathways.

Follow on Funding: None at the time of reporting.

Collaborations: Drs. Amy Smith and Ana Aguilar-Bonilla, Pediatric Neuro-oncologist at Arnold Palmer Hospital for Children/Orlando Health in Orlando Florida. We are now working on a diagnostic test for malignant peripheral nerve sheath tumors for children with NF1 supported by Live Like Bella/FDOH. Two additional clinical collaborators on the project are Drs. Mislen Bauer at Nicklaus Children's Hospital and Dr. Matthew Hall at Miami Cancer Institute, both in Miami.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Matthew D. Hall, MD, MBA

Organization: Miami Cancer Institute, Baptist Health South Florida

Abstract: This year, we are pleased to present the first results from our research. Pediatric brain tumor patients are at high risk of developing neurocognitive deficits following treatment. The perihippocampal subventricular zone contains a niche of radiosensitive neural progenitor cells linked to memory development, and radiotherapy to this brain substructure has been associated with neurocognitive impairment in randomized trials. MRIs in pediatric and young adult brain tumor patients (Age< 35) were prospectively collected at baseline and during follow-up to measure volumetric changes in multiple brain substructures with neurocognitive, laboratory, and quality-of-life assessments. In this planned interim analysis, we model early outcomes for change in hippocampal volume at six months following radiotherapy. Context As of 2/26/2021, 47 patients enrolled on this prospective study and 36 had completed their six-month follow-up assessments after intensity-modulated proton therapy (IMPT). Left and right hippocampus volumes were independently measured on T1 sagittal precontrast MRI at baseline and six-months after radiotherapy using both automated software and physician-delineated contours. The relationship between mean hippocampus dose and change in volume was assessed by Pearson's correlation coefficient. A linear mixed-effects model was applied to evaluate other predictors associated with change in hippocampus volume, assuming random effects of subjects. Potential factors considered were age, gender, tumor location, focal vs. whole brain RT, prior craniotomy, and chemotherapy. Progress to Date Mean hippocampus dose was strongly correlated with change in hippocampal volume at 6 months following radiotherapy ($r=-0.727$, 95% CI [-0.820,-0.596], $p<0.001$). Changes in hippocampal volumes over time were similar between software and physician contours. Hippocampal volume was significantly smaller with mean doses ≥10 Gy (mean Δ -10.8%±5.5%, $p<0.001$), while no significant volume change was observed with mean doses <10 Gy (mean Δ +0.7%±3.9%). In the mixed-effects model, only mean hippocampus dose was significantly associated with hippocampal volume change ($p<0.001$). The final model predicted a -3.4% change in hippocampal volume for every 10 Gy increase in mean dose. The figure illustrates a linear regression of hippocampal volume change on the y-axis vs. Mean Dose to the hippocampus on the x-axis. Impact to Floridians: We concluded that change in hippocampal volume was correlated with hippocampus mean dose at 6 months following radiotherapy. Future analyses will assess volume change in this and other brain substructures over time as a function of radiation dose and will correlate these findings with measured neurocognitive and other late effects. We are really excited by these early results and will be continuing to collect and evaluate other important endpoints, including (1) 12-month/24-month neuroanatomic changes, (2) Endocrine function, (3) Neurocognitive testing scores, (4) patient/parent-reported quality-of-life scores, and (5) exploratory biomarkers. These findings suggest that patients receiving <10 Gy to the hippocampus may be at lower risk for anatomic changes, suggesting that a safer threshold dose may be achieved in some patients to protect against neurocognitive impairments from radiotherapy for brain tumors. This knowledge may help oncologists in Florida and outside our State to protect brain tumor patients from the late effects of our treatments in the future.

Follow on Funding: The Baptist Health South Florida Foundation, $100,000.

Collaborations: Dr. Raees Tonse, a clinical radiation oncology fellow, and Warren Rehrer, BS, a MS3 medical student at Florida International University have received training and worked on this project as part of their training programs at Florida International University and Miami Cancer Institute.

Dr. Matthew Hall (Principle Investigator), Dr. Rupesh Kotecha and Dr. Noah Kalman have enrolled/consented patients for this trial and the study team very much appreciates the participation of these physicians and their patients in our research efforts.

Department of Psychology Laura Gallardo is an undergraduate Psychology student at Florida International University.

Dr. Richard Hamilton, Aileen Moreno, MSW and Benjamin Sieglie, MSW from the Department of Psychology and the Division of Supportive Care Medicine at Miami Cancer Institute have been critical in providing interval neurocognitive testing and evaluations of patients on this trial.

Nicklaus Children's Hospital, Miami, Florida Department of Psychology Dr. Golnar "Goli" Alamdari is a Pediatric Neuropsychology fellow at Nicklaus Children's Hospital who has received training and education as part of this research project and has aided in the interpretation of neuropsychology data from enrolled patients. Dr. Reshma Naidoo has supervised and assisted with considerable neurocognitive testing of patients on this clinical trial.

Department of Pediatric Neurosurgery Dr. Toba Niazi actively participated in review of all MRIs obtained using the automated Neuroquant software in our analysis above to provide quality assurance that the software accurately delineated the hippocampus in all cases.

Department of Pediatric Oncology/Oncology Dr. Ziad Khatib and Dr. Ossama Maher have been particularly instrumental in helping to keep all patients on schedule for their protocol-specified follow-up testing on this study.

Department of Radiology Dr. Nolan Altman and Dr. Santiago Medina have collaborated with our co-investigator from Miami Cancer Institute, Dr. Kevin Abrams, to standardize imaging of patients enrolled on this clinical trial.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Diana Azzam, PhD

Organization: Florida International University

Abstract: This study is a multi-center clinical trial between Florida International University and Nicklaus Children's Hospital. The goal of the study is to evaluate feasibility of providing personalized treatment options based on ex vivo drug screening and genetic testing for pediatric

patients with recurrent and/or refractory cancers. Overall, we have recruited a total of 18 patients into this study and were able to perform drug testing and mutation profiling on 16 patients. The functional drug screening test (DST) panel encompassed 40 formulary drugs frequently used at Nicklaus Children's hospital and 47 non-formulary drugs approved by FDA for cancer treatment as well as drugs from phase III and IV clinical trials. Drug sensitivity score (DSS) was calculated for each drug based on cancer cells' response. DST results were then combined with results from the genetic screen to match actionable mutations with selective targeted therapies. Most importantly, we optimized and successfully performed our drug sensitivity assay on at least 8 different tumor types including leukemia, osteosarcoma, ewing's sarcoma, rhabdomyosarcoma, glioblastoma, astrocytoma, lung, and liver. Fresh tumor samples from 16 patients with ex vivo DST returned between 10-30 treatments options for each patient. These patients showed different responses to the 103 FDA-approved compounds used in the screen. More than half of the evaluated compounds were not active in any of the patients. With these results, 9 patients were treated on assay-guided protocols and 6 have showed clinical objective response, so far. We are currently assessing their progression-free survival and overall response to DST-guided treatment as compared with their own progression-free survival for the most recent regimen on which they had progressive disease. Overall, we have confirmed the feasibility of this methodology in children to identify candidate agents with clinical potential. DST provided valuable information to the oncologists on drug dosing and treatments that may not be effective and should be avoided. We would like to continue to implement this novel and personalized approach to assess clinical response and progression-free survival.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of this reporting.

Journals: Acanda De La Rocha A., Fader M., Coats E., Espinal P., Berrios V., Saghira C., Soto I., Shakra R., Janvier M., Khatib Z., Abdela H., Bittle M., Andrade-Feraud C., Guilarte TR., McCafferty-Fernandez J., Salyakina D. and Azzam DJ. "Clinical Utility of Functional Precision Medicine in the Management of Recurrent/Relapsed Childhood Rhabdomyosarcoma" Under review by JCO Precision Oncology (2021).

Patents: None at the time of this reporting.

Live Like Bella Pediatric Cancer Research Initiative
Appendix Z
Fiscal Year 2020-2021 Completed Grants
Funding Fiscal Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
8LA01	University of Florida	Jonathan Licht, MD.	$200,000	4/30/2021	Yes	Yes	Yes

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

Principal Investigator: Jonathan Licht, MD

Organization: University of Florida

Abstract: The laboratory has studied NSD2, a histone lysine methyltransferase initially identified by its rearrangement and aberrant overexpression in t(4;14)-associated multiple myeloma (MM). Overexpression of NSD2 leads to shifts in chromatin modification, gene expression and cell growth. We described a point mutation (E1099K) in the enzymatic domain of NSD2, present in 10-20% of cases of relapsed pediatric acute lymphoblastic leukemia (ALL). The mutation was found in 1% of the leukemia cells of a child at diagnosis and in 100% at relapse, suggesting that the mutation allows cells to persist in the face of therapy. Preliminary data: The NSD2 E1099K mutation enhanced the rate of H3K36 dimethylation in vitro. Using gene editing we removed the NSD2 mutation from three ALL cell lines. Cell lines harboring E1099K exhibit increased H3K36 dimethylation and reduced H3K27 trimethylation. This led to up-regulation of a set of genes (~400) associated with neural and stromal lineages, not normally expressed in blood cells. This abnormal gene expression program correlates with aggressive biology. Mutant NSD2 cells exhibit reduced apoptosis and enhanced proliferation, clonogenicity, adhesion, and migration. In mouse xenografts, mutant NSD2 cells are more lethal and brain invasive. NSD2 mutant cells were resistant of glucocorticoids commonly used to treat childhood ALL as well as chemotherapy agents.NSD2 or the genes it affects must be targeted to prevent early ALL relapse. Discovery of the mechanism of glucocorticoid resistance conferred by NSD2 mutations in acute lymphoblastic leukemia. Development of assays to functionally determine the critical target genes of NSD2 for aggressive behavior. Showing that EZH2 inhibitors reverse the glucocorticoid resistance of NSD2 mutant ALL and proposal of a clinical trial of exzh2 inhibitors plus dexamethasone to the Children's Oncology Group.

Follow on Funding: Florida Department of Health Targeting the epigenetic modification in glucocorticoid resistance in pediatric acute lymphoblastic leukemia, Jonathan Licht, MD, $250,000.00 Rally Foundation, The role of NSD2 mutation in therapy resistance in childhood ALL, Jianping Li, MD, $50,000.00

Collaborations: Cold Spring Harbor Labs, NY in the construction of a library of guide RNAs directed against NSD2 targets. Richard Lock Children's Cancer Institute Sydney, Australia on patient derived xenogradts from patients with ALL and NCD2 mutation.

Journals: Jianping Li, Julia, Hlavka-Zhang, Jonathan H. Shrimp, Crissandra Piper, Daphne Dupere-Richer, Jacob S. Roth, Duohui Jing, Heidi C. Roman, Catalina Troche, Alok Swaroop, Marta Kulis, Jon Oyer, Christine Will, Min Shen, Alberto Riva, Richard L. Bennett, Adolfo A. Ferrando, Matthew D. Hall, Richard B. Lock, Jonathan D. Licht, PRC2 Inhibitors Overcome Glucocorticoid Resistance Driven by NSD2 Mutation in Pediatric Acute Lymphoblastic Leukemia, Cancer Discovery, (2020).

Patents: None at the time of reporting.