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

2021-2022 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 through the William G. “Bill” Bankhead Jr. and David Coley Cancer Research (Bankhead-Coley) Program, the Live Like Bella Pediatric Cancer Research Initiative (Bella) (both found in section 381.922, Florida Statutes) and the James and Esther King Biomedical Research (King) Program (section 215.5602, Florida Statutes). During Fiscal Year (FY) 2021-2022, $15,439,425 was awarded to Bankhead-Coley, Bella, and King grantees. This funding resulted in 14 Bankhead-Coley, 10 Bella and nine King 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 this annual report. Per statutory requirements, the progress report includes the following information:

- 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.
- 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:
    - A summary of the research project and results or expected results of the research.
    - The status of the research project, including whether the project has concluded or the estimated date of completion.
    - The amount of the grant or fellowship awarded and the estimated or actual cost of the research project.
    - A list of principal investigators under the research project.
    - The title, citation, and summary of findings of a publication in a peer-reviewed journal resulting from the research.
    - The source and amount of any federal, state, or local government grants or donations or private grants or donations generated because of the research project.
    - The status of a patent, if any, generated from the research project and an economic analysis of the impact of the resulting patent.
    - 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.

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 this 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 and treatment options for tobacco-related diseases. The King program funds research initiatives that seek new insights and innovative solutions in the prevention, diagnosis, treatment, and cure of Floridians afflicted by tobacco-related diseases including cardiovascular disease, stroke, lung disease, and tobacco-related cancers, the leading causes of death in Florida and nationally. The long-term goals of the program are to:

- Improve the health of Floridians by researching better prevention, diagnoses, treatments, and cures for cancer, cardiovascular disease, stroke, and pulmonary disease.
- Expand the foundation of biomedical knowledge relating to the prevention, diagnosis,

treatment, and cure of diseases related to tobacco use, including cancer, cardiovascular disease, stroke, and pulmonary disease.

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

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

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

BIOMEDICAL RESEARCH GRANT ADVISORY BOARD OVERVIEW AND MEMBERSHIP

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

- Providing advice on program priorities and emphases.
- Providing advice on the overall program budget.
- Participating in periodic program evaluation.
- Assisting in the development of guidelines to ensure fairness, neutrality, and adherence to the principles of merit and quality in the conduct of the program.
- Assisting in the development of appropriate linkages to nonacademic entities, such as voluntary organizations, health care delivery institutions, industries, government agencies, and public officials.
- Developing criteria and standards for the award of research grants.
- Developing guidelines relating to solicitation, review, and award of research grants and fellowships, to ensure an impartial, high-quality peer review system.
- Reviewing reports of peer review panels and making recommendations for research grants and fellowships.
- Developing and providing oversight regarding mechanisms for the dissemination of research results.
- 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 July 2022, are listed below. There is currently one vacancy. (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

Guilherme Oliveira, MD, MBA, Professor of Medicine, Vice-President and Chief, Heart and Vascular Institute, and Chief of the Division of Cardiovascular Sciences, Tampa General Hospital; Seat: House of Representatives

Srikumar P. Chellappan, PhD, Professor and Chair, Department of Tumor Biology, Moffitt Cancer Center Seat: House of Representatives

Vacant Seat: American Lung Association

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 Florida Department of Health's (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).

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

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

FUNDING CYCLE FOR FISCAL YEAR 21-22

Awards were finalized to support the following research priorities for Bankhead-Coley, King, and Bella initiative grants:

23 Awards – Prevention and Treatment: (12 Bankhead-Coley, 5 King, and 6 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.

2 Awards – Technology Transfer: (1 Bankhead-Coley and 1 King) The goal 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.

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

0 Awards – Tobacco Use, The Impact of Obesity

1 Award – Screening: (1 King) 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 King and 2 Bella) 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, Central Nervous System, reproductive, developmental impairment, graft-versus-host disease).

1 Award – 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 can be part of a multicenter clinical trial.

Figure 1: Bankhead-Coley Applications and Funded Projects

For FY 2021-22, 105 applications were submitted in response to the Bankhead-Coley funding opportunity announcement and 14 cancer research projects were awarded.

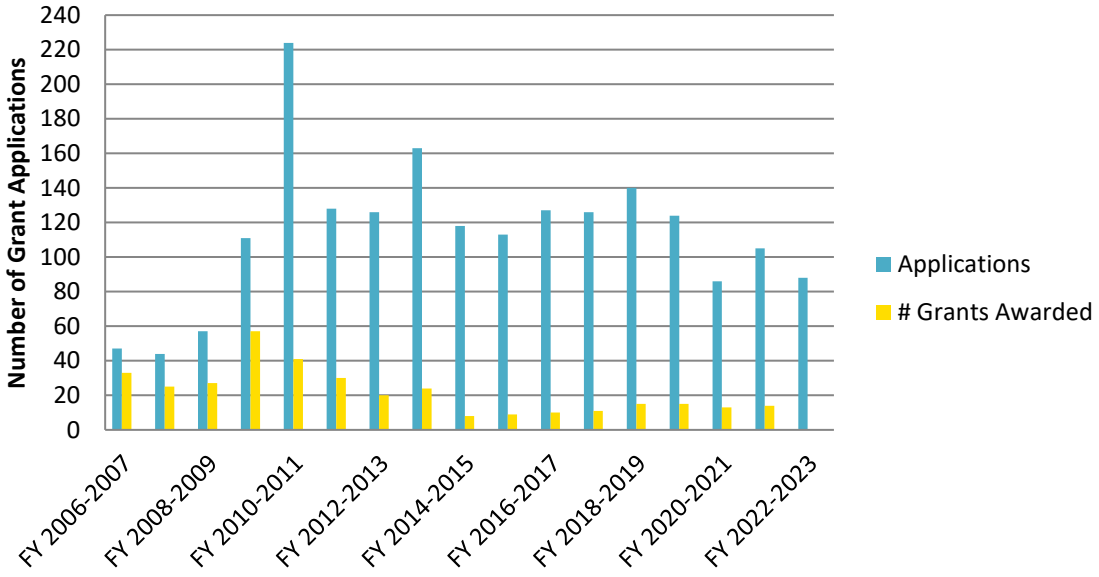

Figure 2: Bella Applications and Funded Projects

For FY 2021 - 2022, 15 grant applications were submitted in response to the Bella funding opportunity announcement, and 10 pediatric cancer research projects were awarded. As the program continues to become known, it is anticipated that more grant applications will be submitted.

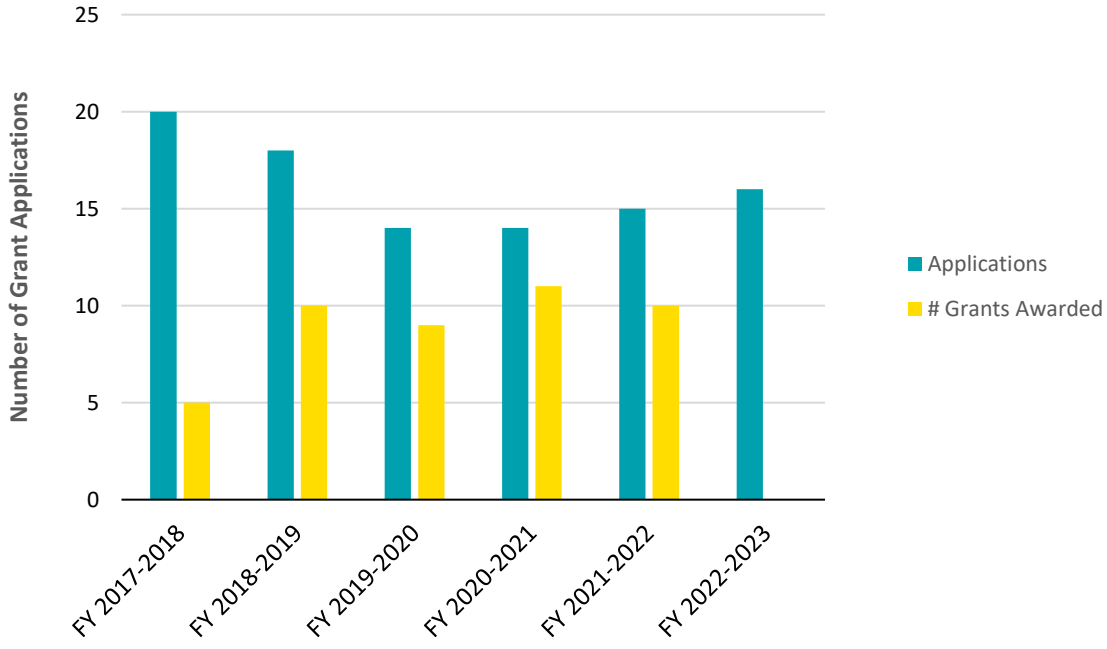

Figure 3: King Applications and Funded Projects

For FY 2021-22, 59 applications were submitted in response to the King funding opportunity announcement and nine tobacco-related disease research projects were awarded.

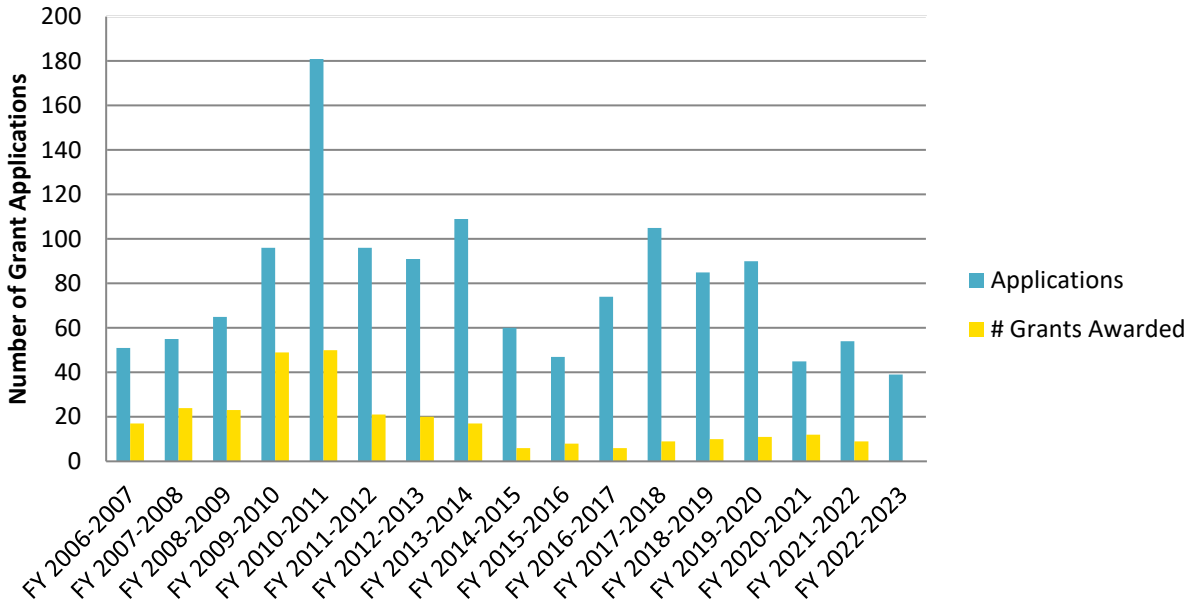

Table 1: Awarded Institutions 2006-2022

The State of Florida research infrastructure for cancer and tobacco-related diseases continues to expand over time. The following research institutions have received research funding to create and build the research infrastructure.

All Children's Research Institute	Florida State University	Saneron CCEL Therapeutics
Ave 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

NATIONAL INSTITUTES OF HEALTH (NIH) RESEARCH FUNDING AND FLORIDA'S RANKING

For the past five years, the state of Florida has remained 12th in the US for the total amount of federal funding awards. There was an increase in the total amount of funding for FY 2021-2022. NIH funding for Florida has increased to over $780 million. These results reflect Florida's initiative to expand upon research to improve scientific understanding of various diseases and health disparities.

Figure 4: NIH Funding for Florida FYs 2014 - 2015 through 2021 - 2022

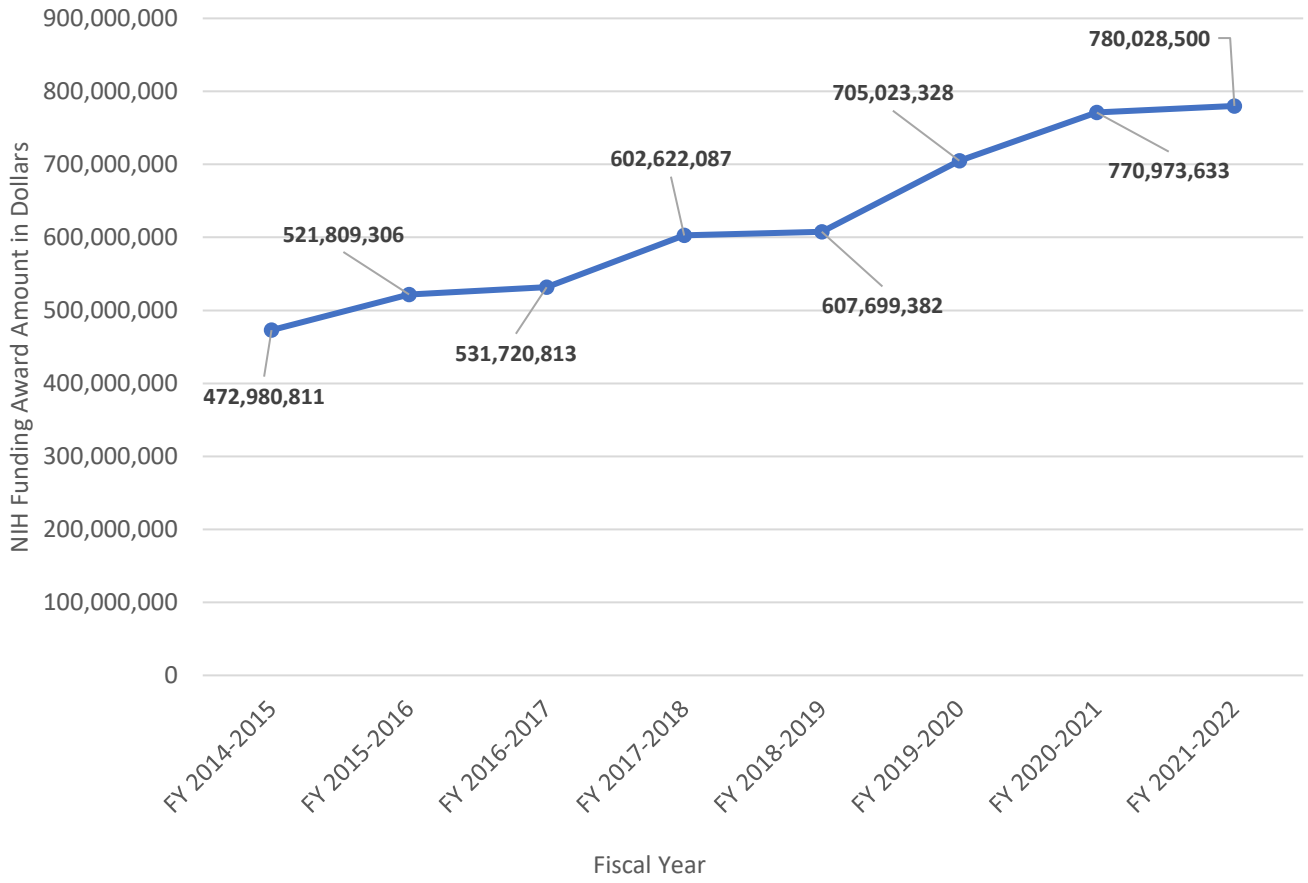

Table 2: NIH Research Funding to the Top 20 States for FY 2021 - 2022

NIH Biomedical Research State Funding and Rankings FY 2021 – 2022		
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	$780,028,500	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

(*Source: NIH Research Portfolio Online Reporting Tools (RePORT)*

William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program

Appendix A

Fiscal Year 2021-2022 Newly Awarded Active Grants

Funded Fiscal Year 2021-2022

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
22B01	H. Lee Moffitt Cancer Center and Research Institute	Susan Vadaparampil, PhD	$1,424,806.00	9/30/26	No	No	No
22B02	H. Lee Moffitt Cancer Center and Research Institute	Eric K. Lau, PhD	$573,000.00	3/31/25	No	No	No
22B03	H. Lee Moffitt Cancer Center and Research Institute	Jose R. Conejo-Garcia, MD, PhD	$1,432,499.00	9/30/26	No	No	No
22B04	H. Lee Moffitt Cancer Center and Research Institute	Inna Smalley, PhD	$287,520.00	3/31/25	No	No	No
22B05	H. Lee Moffitt Cancer Center and Research Institute	Joseph Kissil, PhD	$573,000.00	3/31/25	No	No	No
22B06	H. Lee Moffitt Cancer Center and Research Institute	Brandon Manley, MD	$716,250.00	9/30/26	No	No	No
22B07	H. Lee Moffitt Cancer Center and Research Institute	Vincent Luca, PhD	$573,000.00	3/31/25	No	No	No
22B08	Mayo Clinic Jacksonville	E. Aubrey Thompson, PhD	$573,000.00	6/30/25	No	No	No
22B09	Nova Southern University	Dmitriy Minond, PhD	$573,000.00	3/31/25	No	No	No
22B10	University of Florida	Jonathan Licht, MD	$573,000.00	3/31/25	No	No	No
22B11	University of Florida	Guangrong Zheng, PhD	$100,000.00	10/31/22	No	No	No
22B12	University of Miami	Antonio Barrientos, PhD	$573,000.00	3/31/25	No	No	No
22B13	University of Miami	Jonathan Schatz, MD	$573,000.00	3/31/25	No	No	No
22B14	H. Lee Moffitt Cancer Center and Research Institute	Alvaro Monteiro, PhD	$200,000.00	09/30/2024	No	No	No

1. **Grant #:** 22B01 HPV MISTICS: HPV Multilevel Intervention Strategies Targeting Immunization in Community Settings

Principal Investigator: Susan Vadaparampil, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: Human Papillomavirus (HPV) vaccination is a safe and effective strategy to reduce multiple cancers. Men and women in Florida suffer from HPV-related cancers at higher rates than the nation; yet only 56% of 13-17-year-olds are up to date with HPV vaccination, representing a significant missed opportunity to reduce HPV-related cancer. Evidence-based strategies to improve HPV vaccine rates exist but Florida physicians' use of these strategies is low. Utilizing an innovative partnership with Health Choice Network (HCN), this project features a Hybrid Type 1 effectiveness-implementation, stepped-wedge randomized controlled trial design assessing effectiveness of HPV Multilevel Interventions Strategies Targeting Immunization in Community Settings (HPV MISTICS) in federally qualified health centers (FQHCs). HPV MISTICS uses interventions at provider, parent, and system levels. The provider intervention is a 1-hour online training led by a Physician Educator on how to utilize the Announcement Approach (presumptive recommendation) to recommend adolescent vaccinations. The parent intervention includes a pre-visit HPV vaccine notification postcard. The system intervention involves training a Vaccine Champion in each FQHC on use of Florida's statewide immunization registry (Florida SHOTS) to monitor HPV rates, generate individual monthly reports for providers on their patient panel's HPV vaccination uptake, and implement reminder/recall to notify patients about HPV vaccine doses. The proposed aims are 1) test whether HPV MISTICS increases HPV vaccine initiation and completion rates in adolescents 11-17 2) explore covariates of intervention effects and 3) explore equity of implementation

outcomes and identify implementation barriers and facilitators. The study team has gained approval by Moffitt's Scientific Review Committee and Institutional Review Board (MCC #21902) and have begun conducting regular meetings and with study partner, HCN. HCN has assisted in identifying which of their FQHC systems will participate. Seven FQHCs are confirmed, with one more pending approval by the FQHC's Scholarly Activity Committee. HCN has shared baseline HPV vaccination rate data with the study team. The research team has begun compiling evidence-based content to be included in HPV Vaccine Champion trainings and are working to draft preliminary Vaccine Champion training slides and materials.

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 #:** 22B02 The Trouble with Testosterone: Delineating How Androgen Drives Melanoma Invasiveness and Metastasis Via Fucosylation-Regulated Cellular Adhesion

Principal Investigator: Eric K. Lau, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: Melanoma incidence and mortality rates are higher for men, with approximately 50% more new cases and twice the lethality than women in the US in 2021. The male sex hormone androgen/testosterone has been reported to increase melanoma progression, and the research team found that androgen induces melanoma proliferation and invasiveness. Thus, inhibition of androgen signaling represents an attractive new treatment strategy for melanoma, given the clinical availability of androgen receptor (AR) antagonists (ARas) for prostate cancer (PCa). However, the researchers lack crucial mechanistic insights and biomarkers for informing which pathological/therapeutic contexts—which melanoma patients—would benefit from inhibition of AR. Thus, studies elucidating key androgen-/AR-regulated mechanisms driving melanoma pathogenesis are urgently needed.

The reserach team discovered a significant mechanistic connection between AR and melanoma fucosylation (protein modification by the dietary sugar L-fucose). These data indicate that AR signaling induces tumorigenic fucosylation to drive melanoma pathogenesis by altering cell:cell adhesion structures and increasing invasiveness. The ability to block invasiveness using already clinically approved ARas will significantly and quickly change treatment paradigms for melanoma.

Here, the research team will determine how androgen/AR regulates melanoma invasiveness and potential correlations of AR/fucosylation/adhesion structure signatures with clinical parameters. The team will test the hypothesis that AR upregulates fucosyltransferase 4 (FUT4), which alters adhesion to drive melanoma invasiveness and metastasis in 2 Specific Aims (SAs):

In SA1, the team will determine how (i) FUT4 regulates key adhesion structures, (ii) FUT4-adhesion signaling regulates melanoma invasiveness (in vitro cell-based assays using cells manipulated for FUT4/adhesion component(s)). In SA2, the aim is to determine how AR-FUT4-regulated adhesion drives melanoma metastasis in vivo and which patients might benefit from

ARas (mouse models using ARas and melanoma cells in SA1; patient tissue & expression analyses).

The research team expects to improve melanoma patient outcomes by elucidating a new pathogenic mechanism, the targeted inhibition of which is highly feasible with ARas currently used to treat PCa. These findings help identify which patients will benefit from this new treatment strategy. Return on investment is expected to be high and timely.

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 #:** 22B03 Heterogeneity of Metastatic Small Cell Lung Cancer; Implications for the Design of Effective Immunotherapies

Principal Investigator: Jose R. Conejo-Garcia, MD, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: Small cell lung cancer (SCLC) is an aggressive malignancy, but SCLC is characterized by many mutations, which provides a rationale for designing immunotherapeutic interventions. Accordingly, combined immune checkpoint blockade (a form of immunotherapy) has recently elicited objective responses, while immunotherapy after chemotherapy increased overall survival. Nevertheless, SCLC remains a major clinical challenge due to presentation at advanced, metastatic stages, and rapid progression. Aggressive malignant progression and spreading has also limited a clear understanding of the progression of the human disease, and the design of more effective treatments as a result. To address these barriers and move the field forward, Moffitt Cancer Center has established a Rapid Tissue Donation (RTD) program that provides timely access to the entire repertoire of metastatic lesions in terminal patients, who generously donate their tissues for this research. Using this unique resource, research project staff plan to characterize the barriers that impair the effectiveness of immunotherapies, with a focus on the heterogeneity of the disease, and novel interventions to overcome these hurdles. Based on the expertise of research project staff on tumor immunology and clinical immunotherapy, as well as access to unique clinical specimens provided through an effective Rapid Tissue Donation program, research project staff postulate that the effectiveness of immunotherapies against small cell lung cancer is thwarted by heterogeneous immunogenicity across different tumor masses, along with immune cell sequestration and metabolic restrictions at tumor beds. Based on preliminary results, the central hypothesis of this project is that pleural effusions and tumor-infiltrating immune cells that “snap” chunks of tumor cell membranes (“trogocytic” T cells) will provide a source of effector lymphocytes able to target multiple tumor masses; provided that metabolically-driven cell stress pathways are co-targeted. Research project staff proposes the following Specific Aims: Aim 1. Define intra-and inter-tumor heterogeneity in the immunogenicity of human small cell lung cancer. Aim 2. Elucidate the trajectory of differentiation of tumor-reactive T cells in small cell lung cancer. Aim 3. Design cellular therapies that target heterogenous metastatic disease. Insight derived from these approaches will, first, define the role and heterogeneity of neoantigens and tumor

microenvironmental immune cells in small cell lung cancer; a poorly characterized disease, due in part to its aggressiveness. Most importantly, research project staff will provide the field with a mechanistic rationale for more effective immunotherapies that target the diversity of this human 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.

4. **Grant #:** 22B04 Defining and Targeting the Immune-suppressive Metabolic Microenvironment of Leptomeningeal Melanoma Metastases

Principal Investigator: Inna Smalley, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: Florida is second in the nation for the highest rates of melanoma cases, the deadliest form of skin cancer. Despite the remarkable progress in the development of targeted and immune therapies against advanced melanoma, most patients ultimately relapse. One of the most serious complications of advanced melanoma is spread of cancer cells to the leptomeninges and their infiltration into the cerebrospinal fluid (CSF), collectively known as leptomeningeal melanoma metastasis (LMM). The leptomeninges and CSF are uniquely immunosuppressive and are emerging as a more frequent, common site of melanoma progression. Clinically, patients with LMM have very short survival times regardless of treatment (typically 8-10 weeks) and no FDA-approved therapies exist for these patients. There is an urgent clinical need to better understand the biology of LMM and to identify LMM-specific therapeutic targets. The goal of this proposal is to improve understanding of leptomeningeal melanoma metastasis and identify therapeutic vulnerabilities for this disease. In preliminary single-cell transcriptomic analysis of LMM, and melanoma metastases from the brain and skin, the researchers have identified major differences in potential metabolic programs of the tumor specific to the leptomeninges. Furthermore, the research team found significantly higher proportions of infiltrating macrophages with a transcriptional signature associated with an alternative, pro-tumorigenic phenotype. Traditionally, metabolism has been viewed as a collection of catabolic and anabolic pathways that generate energy and biosynthetic precursors required for growth and survival. However, emerging evidence suggest broader roles for metabolic processes in controlling other aspects of physiology, including immune cell functions. The researcher tema believes that the metabolic adaptations melanoma cells undertake to survive in the unique microenvironment of the leptomeninges alter the metabolism of infiltrating immune cells, leading to an increase in immune tolerance and tumor survival. The research team will define the main metabolic adaptations that drive the immune suppressive environment in LMM-resident macrophages and identify the ideal axis for therapeutic intervention that inhibits LMM progression. A ground-breaking integrated single-cell metabolomics and RNAseq approaches will be utilied, paired with tracing of metabolic flow to examine how LMM-specific changes in melanoma metabolism impact the alterations of key metabolic pathways of macrophages. The research team will then utilize innovative cell cultures and animal models of LMM to test if targeting LMM-specific metabolic adaptations in melanoma cells or the LMM-driven metabolic remodeling of macrophages will attenuate the immune- suppressive

environment and inhibit LMM progression. This data will lay the groundwork the development of clinically relevant therapeutic approaches for LMM.

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 #:** 22B05 Establishing the Functional Differences Between Variant Oncogenic Kras Alleles and Identification of Allele-selective Inhibitors

Principal Investigator: Joseph Kissil, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: The rat sarcoma virus (RAS) proteins (HRas proto-oncogene (HRAS), KRAS proto-oncogene (KRAS), and NRAS proto-oncogene (NRAS) are small G-proteins that function as master regulators of signaling pathways conveying stimuli from the extracellular environment that impact cellular behaviors such as proliferation, death and differentiation. Small G-proteins are the most frequently mutated oncogenes in cancer, with mutations found in approximately a third of all cancers. The KRAS gene is the most frequently mutated with mutations found in pancreatic carcinomas (>90%), lung adenocarcinomas (>30%) and colorectal tumors (>40%). In these tumors, the activity of the oncogenic KRAS protein is required for the proliferation and/or survival of the tumor cells and thus represents a high-value target for therapeutic development. Extensive efforts to target the RAS proteins, have been ongoing but have proven to be challenging due to multiple reasons stemming from the biology of the RAS proteins, complexity of downstream effector pathways and upstream regulatory networks. An important lesson learned from research over the past several years is that not all mutant RAS alleles are created equal. Previously, researchers thought that the major mutations in the RAS proteins, those altering amino acids G12, G13 or Q61, all function in a similar manner to impair guanosine triphosphate(GTP)ase activity, thus resulting in the balance of cellular RAS being in the GTP-bound, active (ON) state. However, several studies clearly demonstrate that different RAS alleles lead to different functional outcomes and sensitivities. These findings underscore the potential for identifying specific biology associated with each of the different oncogenic RAS alleles and exploiting this therapeutically, as demonstrated by recent successes with development of small-molecules directly targeting the oncogenic KRAS-G12C variant. The researchers' long-term goals are to understand the functions of the different RAS alleles and identify specific vulnerabilities that can be exploited for therapeutic gain. Towards this goal the researchers will focus on mutations in KRAS using a newly developed allelic series of isogenic lung adenocarcinoma (LuAD) cells. This series, which consists of a panel of isogenic LuAD cells that differ only in the status of KRAS at codon G12 or G13, was created using CRIPSR-based editing, and will allow the researchers to carefully characterize and compare the effects of these mutations at a cellular level, permitting elucidation of the differential signaling events downstream of the oncogenic KRAS variants using novel transcriptomic and proteomic approaches. Importantly, researchers will employ additional innovations that have already shown to dramatically improve discovery of small molecules that are selective against oncogenic KRAS. These include the use of a 3D spheroid-based screening format, which the researchers have previously shown to expose new vulnerabilities that would not have been

identified in traditional 2D format assays. Finally, cutting-edge approaches for target deconvolution will be employed by functionalizing the identified screening hits with diazirine and alkyne moieties for in-cell UV crosslinking and click chemistry for target pull-down respectively, where cross-linked targets will be identified by mass spectrometry. These efforts will greatly facilitate target identification and determining the mechanism of action of the identified hits.

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 #:** 22B06 Establishing the Role of Aberrant Splice Variants as a Clinical Biomarker In Clear Cell Renal Cell Carcinoma

Principal Investigator: Brandon Manley, MD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: This proposal is an innovative study that seeks to evaluate the role of specific molecular splicing events that frequently occur with the most common type of kidney cancer, clear cell renal cell carcinoma (ccRCC). The research team is investigating the role of this novel splice variants as biomarkers in patients with locally advanced disease who have a high chance of recurrence. Splice variants have demonstrated clinical applicability in treating patients in several solid malignancies, including lung cancer, prostate and brain cancer but their role or impact among ccRCC remains unknown. Building on the strong scientific rationale of the team's previous studies, this proposal will produce several impactful short-term results. A candidate list of 6 aberrant splice variants are ideal biomarkers for ccRCC given their specificity, high frequency of occurrence in ccRCC tumors. Additionally, these molecular events happen in the same location of selected candidate genes in all ccRCC patients making those patients an excellent target for assay development. The results of this study will define the ability of these aberrant splice variants to be detected in the plasma of patients with ccRCC and how their presence or absence after surgery may predict detect early recurrence of disease. Successful results of this study would overcome critical steps to the first blood-based clinical biomarker test for kidney cancer patients. The researchers have designed this proposal in such a fashion that successful detection of the splice variants in the plasma of patients using digital polymerase chain reaction (dPCR) will allow for rapid integration to the clinic. This technology is highly sensitive, economically practical for large scale testing and already employed for clinical use to identify alterations in genes such as epidermal growth factor receptor (EGFR) gene and the B-Raf proto-oncogene (BRAF). Furthermore, several results drawn from this proposed research could have long-term impacts on the field of kidney cancer in its goal to improve patient survival. Upon demonstrating the clinical role of these biomarkers and development of an accurate testing assays, future studies can further refine its ability to guide systemic treatment strategies. The researchers previously published data shows a possible correlation with poor response to immunotherapy in those patients whose tumor have a splice variant of EGFR. Development of personalized therapy strategy is important since there remains little comparative data from currently approved drugs, a wide diversity of drug mechanisms and an ongoing emergence of combination treatments that lack clarity on which patients should receive a specific treatment. Additionally, by demonstrating the clinical applications of splice variants research can be

expanded to better understand the biological repercussions of these alterations and how these changes may be further therapeutically exploited. Lastly, by demonstrating impact in locally advanced ccRCC patients the researchers will be well positioned to investigate the possible role of aberrant splice variants in the early detection or screening for ccRCC, especially in high-risk populations. These outcomes synergize with the ultimate goal of extending the life expectancy and eliminating the disease-related deaths of Florida residents and others with kidney 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.

7. **Grant #:** 22B07 Structure-guided Engineering of Lag3 Immunomodualtory Function

Principal Investigator: Vincent Luca, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: Over the past several years, immunotherapy has emerged as a highly effective treatment for cancer. In contrast to chemotherapy, which kills cancer cells with toxic chemicals, immunotherapy teaches a patient's immune system to eradicate tumors. As current immunotherapy treatments are only successful in approximately 30% of cases, scientists are actively searching for ways to create new classes of immunotherapy drugs. The researchers are using two different methods to guide the development of next-generation immunotherapies. The first strategy is to use a high-resolution imaging technique called x-ray crystallography to "see" how the receptor lymphote activation gene 3 (LAG3) sends signals that suppress immune cell function. By visualizing LAG3 molecules on the atomic scale, the goal is to obtain molecular blueprints that inform the design of more effective drugs. For the second strategy, the researchers will harness these blueprints to engineer decoy proteins that can block incoming signals to the LAG3 receptor. These decoys will then be used to block LAG3 from shutting down the immune response. Initially, the LAG3 decoys will be used to re-activate T cells in a laboratory setting. However, if these tests are successful, the long-term goal is to proceed to clinical trials in melanoma 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.

8. **Grant #:** 22B08 Spatial Analysis of the Immune Landscape of Stage 4 Triple Negative Breast Cancer

Principal Investigator: E. Aubrey Thompson, PhD

Organization: Mayo Clinic Jacksonville

Abstract: Locally advanced or metastatic triple negative breast cancer (Stage 4 TNBC) is a devastating disease with near 100% mortality. Standard of care for previously untreated Stage 4 TNBC involves immuno-therapy with an antibody against the immune checkpoint inhibitor PD-L1 (atezolizumab or pembrolizumab) plus chemotherapy (usually a taxane). A subset of patients who receive such therapy have clinical benefit, defined as stable disease or tumor regression for at least six months. However, about half of the patients receive no benefit and progress rapidly with lethal consequences. The clinical challenges are 1) to understand why some patients benefit, 2) to Identify those patients who are unlikely to benefit, and 3) to develop alternative therapeutic strategies for such patients. The research team is motivated by the core concept that a rational approach to immuno-therapy of TNBC requires a detailed understanding of the numbers, types, activities, and location of immune cells within the tumor mass. In pursuit of this objective, the team has played a major role in the development of NanoString GeoMx digital spatial profiling (DSP) technology. The researchers laboratory was one of four academic sites, world-wide, that was selected for beta testing of this technology; and has, to date, processed >1600 samples, mostly breast cancer. The research team is confident that this research team has more experience with this technology than any laboratory outside of NanoString.

DSP technology involves multi-plex digital spatial quantification of antibody binding to a single 5-micron formalin-fixed, paraffin-embedded (FFPE) section. The dynamic range is at least 5 logs, and the output is digital: the number of antibody molecules bounds is counted and, from those counts, infer spatially defined abundance of 81 key immune and other target proteins. GeoMx technology has recently been developed for measuring spatially defined abundance of 18,000 transcripts (WTA: whole transcriptome analysis) in a single 5-micron FFPE section. This technology therefore enables the team, for the first time, to measure spatial multiplex protein and transcript profiles and to define relationships between therapeutic outcome and these features in TNBC.

The research team has recently completed DSP analysis of 319 early stage TNBC tumors. Four highly interactive key features were identified as associated with good prognosis: 1) intraepithelial antigen-presenting activity (APC); 2) intraepithelial T cell activation status (TCA); 3) intraepithelial PD-L1 abundance; and 4) intraepithelial IDO1 abundance. The team will carry out DSP analysis to test the hypothesis that these makers, APC, TCA, PD-L1 and IDO1 alone or in combination, are associated with clinical benefit in Stage 4 TNBC patients who receive anti-PD-L1 immunotherapy plus chemotherapy. A secondary analysis will involve whole transcriptome spatial analysis to identify targetable features that may be exploited to improve clinical benefit in patients who do not benefit from PD-L1 plus chemotherapy.

The study goals are to provide a comprehensive spatial analysis of the immune landscape of Stage 4 TNBC, identify features that may guide therapeutic decision making in patients who present with Stage 4 disease, and identify potential therapeutic targets that can be exploited for management of advanced TNBC patients who fail standard of care anti-PD-L1 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.

9. **Grant #:** 22B09 Spliceosomal Modulation for Regulation of Melanoma Immunogenicity

Principal Investigator: Dmitriy Minond, PhD

Organization: Nova Southern University

Abstract: As estimated by the National Cancer Institute (NIH/NCI), there are more than 900,000 people living with melanoma in the US. In Florida, approximately 700 people die from melanoma every year and >7000 new cases are diagnosed every year (http://www.flhealthcharts.com), which makes melanoma treatment one of the top research priorities in Florida. Despite recent advances in melanoma drug discovery, the average overall survival of patients with late-stage metastatic melanoma is approximately three years. Instances of complete response are very rare; therefore, more life-prolonging therapies are needed. This suggests a need for new approaches and targets for melanoma drug discovery. The objective of this proposal is to determine the role of spliceosomal proteins hnRNPH1 and H2 (H1 and H2, 96% homology) in melanoma immunogenicity, which could lead to the novel approaches to therapy, which is one of the research priorities set forth by Florida Biomedical Research Advisory Council. The study preliminary findings suggest that small molecule modulation of spliceosome can lead to the increase of melanoma cell immune signaling, which can be beneficial to the patients. The researchers are proposing the following specific aims: (1) Determine role of H1/H2 in melanoma immunogenicity in vitro; (2) Determine role of H1/H2 in melanoma immunogenicity in vivo; and (3) Determine in vivo efficacy of spliceosomal modulation in combination with immunotherapy. This team is uniquely positioned to successfully execute the Aims of this study. Drs. Venkatesan and Velayutham provide expertise in animal and molecular studies. Overall, these proof-of-principle studies will provide evidence of role of H1/H2 in melanoma immunogenicity and will form a basis for further studies to assess its potential for 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 #:** 22B10 Mitochondrial Modulators of Multiple Myeloma Growth and Therapy Resistance

Principal Investigator: Jonathan Licht, MD

Organization: University of Florida

Abstract: Multiple myeloma (MM), an incurable blood malignancy of antibody producing plasma cells, is of great importance to Florida due to its association with aging and much higher incidence among black populations. Despite introduction of new therapies, most patients relapse due to drug resistance. MM arises in part from acquired chromosomal anomalies that yield high-level expression of cancer-causing genes. Most therapies used for MM target malignant cells based upon their plasma cell nature and do not target specific mutations or translocations. Chromosomal translocation t(4;14), found in 15% of MM, leads to overexpression of the histone methyltransferase NSD2 which drives an oncogenic gene expression program associated with poorer prognosis. Identification of genes required for MM

cell growth, including high-risk subtypes like t(4;14), is a new way to begin development of novel therapies.

Through a gene disruption fitness screen in NSD2 high and low MM cells, the research team has identified genes whose loss is more detrimental to NSD2 high cells. Among these was the gene encoding adenylate kinase 2 (AK2). The Broad dependency map database indicates that only a few cell types require AK2 for growth, including MM with t(4;14). AK2, localized in the mitochondrial intermembrane space, catalyzes the reversible reaction adenosine diphosphate (ADP) + ADP = adenosine monophosphate (AMP) + ATP. High antibody production MM puts cells under endoplasmic reticulum (ER) stress, increasing the need for energy (ATP) to fold proteins. The researchers' initial data suggests that AK2 is indeed required to resolve ER stress in MM cells, possibly due to its ability to generate ATP. Rapid AK2 depletion activates unfolded protein response (UPR) cell death signaling. Analysis of Multiple Myeloma Research Foundation and the researchers own data indicate that AK2 overexpression is linked to MM resistance to proteasome inhibitors, therapies that kill MM by generating ER stress. The research team hypothesizes that MM growth depends on mitochondria energy production and AK2 to prevent ER stress, representing a therapeutic vulnerability. Therefore, the study aims will be: Aim 1: Define the molecular basis of the dependence of NSD2 high MM on AK2. The research team will determine how NSD2-mediated changes in gene expression affects mitochondrial metabolism and dependence on AK2. NSD2 overexpression may prevent metabolic adaptation to AK2 depletion. The team will determine how altered apoptotic UPR signaling in NSD2-high MM cells affects susceptibility to AK2 loss. Aim 2: Characterize the role of AK2 in MM in vitro and in vivo by determining the effect of AK2 depletion on proliferation, migration, invasion, and apoptosis in cell culture and mouse models. The effect of AK2 disruption on mitochondrial function, cell metabolism and gene expression in MM will be ascertained. Aim 3: Investigate the role of AK2 and other mitochondrial constituents in MM fitness and therapy response within the bone marrow microenvironment. The effect of AK2 knockdown and overexpression on the ability of MM cells to form spheroids in a 3D model resembling bone marrow will be investigated. The 3D culture will be used to determine how AK2 affects sensitivity of MM cells to therapeutic agents. The role of AK2 in modulating MM growth and response to PIs will be assessed in vivo using the MOPC315.BM syngeneic mouse model. Functional screens of nuclear-encoded mitochondrial genes will be performed in vivo to elucidate the role of mitochondria in MM progression and response to PI 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.

11. **Grant #:** 22B11 The Role of Immune Microenvironment in Small Cell Lung Cancer

Principal Investigator: Guangrong Zheng, PhD

Organization: University of Florida

Abstract: Lung cancer is the leading cause of cancer-related death in the US and worldwide, among both men and women. Lung cancer claims more lives than do colon, prostate, ovarian and breast cancers combined each year. Small cell lung cancer (SCLC) that accounts for

around 15% of lung cancer cases is the most aggressive subtype of lung cancer with a five-year survival rate of less than 5%. The results of numerous clinical trials have been disappointing and to date no approved targeted-therapy for small cell lung cancer is available. As a result, treatment options for small cell lung cancer have not had the same progress as non-small cell lung cancer (NSCLC). Immunotherapy such as anti-programmed death- 1 (PD1) and anti-PD-L1 antibodies that boost immune system to eliminate cancer cells has demonstrated unprecedented clinical activity in several difficult-to-treat cancers including non-small cell lung cancer but has only showed modest efficacy in small cell lung cancer. The immunotherapy drug durvalumab and atezolizumab (anti-PD-L1 antibody) have recently received FDA approval as a first line therapy. Compared with chemotherapy alone, however, adding durvalumab or atezolizumab only extends patient median overall survival by two months. Such modest efficacy of immunotherapy drugs observed in small cell lung cancer highlights the unmet need for more effective combination therapy approaches. The mechanistic basis for this impaired anti-tumor immunity in small cell lung cancer remains unknown but mounting evidence suggests that the tumor microenvironment plays a key role in determining the efficacy of immunotherapy. Dendritic cells (DC) are professional antigen-presenting cells that play a key role in orchestrating immune responses against tumor development. However, various immunosuppressive factors in the tumor microenvironment undermine DC function. Importantly, immune dysfunctional DCs result in uncontrolled tumor progression, indicating that maintaining the immune competence of DC is critical for successful anti-tumor immunity. It has long been suggested that accumulation of lipids in the tumor microenvironment (TME) drive DC dysfunction. The underlying mechanism, however, is unexplored. In this proposal, the research team hypothesizes that lipid-laden DCs in the tumor microenvironment are induced by tumor-derived exosomes (TDEs), small vesicles released by tumor cells. Researchers uncover that TDE-derived long-chain fatty acids critically contribute to lipid accumulation and consequently dysfunction of DCs in SCLC. DCs uptake TDEs with large amount of fatty acids that activates peroxisome proliferator activated receptor a (PPARa) signaling, a master regulator involved in lipid metabolism. The activation of peroxisome proliferator activated receptor alpha (PPARa) in DCs further leads to aberrant lipid accumulation, which culminates in the induction of immunosuppressive enzyme arginase 1 (Arg1) and consequently dysfunction in DCs. Importantly, inhibition of PPARa effectively correct the immune dysfunction of DCs, and enhanced anti-tumor efficacy of immunotherapies in SCLC. Collectively, these findings indicate that TDEs, as fatty acid carriers, adversely affect DCs function, and that targeting PPARa could be a novel therapeutic strategy for small cell 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.

12. **Grant #:** 22B12 Targeting Mitochondrial Protein Synthesis to Combat Blood Malignancies

Principal Investigator: Antonio Barrientos, PhD

Organization: University of Miami

Abstract: Targeted signaling inhibitors for hematologic malignancies may lead to limited clinical efficacy due to the outgrowth of subpopulations with alternative pathways independent of the

drug target. Relapse/refractory disease that results from treatment with targeted signaling inhibitors is a major hurdle in obtaining curative responses. Interestingly, work over the past decade or more has shown that chronic myelogenous leukemia (CML) stem cells (CD34+CD38-) are resistant to targeted signaling inhibitors, such as the BCR-ABL kinase class of inhibitors, often a problematic source of resistance leading to minimal residual disease. Recent studies have shown that some forms of lymphoma and leukemia cells have an energy metabolism highly dependent on mitochondrial oxidative phosphorylation. Tigecycline, a US FDA-approved antibiotic, inhibits the synthesis of mitochondrion-encoded proteins due to the similarity of bacterial and mitochondrial ribosomes, leading to selective lethality in hematologic malignancies reliant on enhanced oxidative phosphorylation. Indeed, it was established that CML stem cells are reliant on upregulated oxidative phosphorylation and a combination of imatinib and tigecycline could eradicate therapy-resistant CML, both in vitro and in animal models. The main goal of this proposal is to determine the mechanism by which elatol inhibits mitochondrial translation and its usefulness to target mitoribosomes as a therapeutic strategy against several types of leukemia. The researchers hypothesize that the dependence of leukemia cells on OXPHOS especially creates a vulnerability to inhibition of mitochondrial protein synthesis. Two specific aims to test this hypothesis have been developed: Aim 1. Characterize the general metabolomics flux and specifically mitochondrial energy metabolism, gene expression, and OXPHOS system organization in an array of leukemia and lymphoma cell lines in comparison with healthy bone marrow and blood cell lines. Aim 2. Determine the sensitivity of oxidative and glycolytic leukemia and lymphoma cells to pharmacological interference of mitochondrial protein synthesis by using classical and new drugs (elatol) that act as mitoribosome inhibitors, alone or in combination with imatinib or other currently used therapeutic agents. It is anticipated that these studies will shed light on the energetic metabolism of leukemia cells and will determine the suitability of genetic and pharmacological interventions that target mitochondrial translation to eliminate leukemia cells selectively.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

13. **Grant #:** 22B13 Inhibition of the Cell-cycle Kinase GAK, a Novel Therapeutic Target in Diffuse Large B-cell Lymphoma

Principal Investigator: Jonathan Schatz, MD

Organization: University of Miami

Abstract: New treatments are needed for patients with diffuse large B-cell lymphoma (DLBCL), an aggressive blood cancer diagnosed in nearly 3,000 Floridians annually. The research team performed a specialized screen for new drug targets and discovered that inhibiting the enzyme cyclin-G associated kinase (GAK) is a promising strategy for attacking DLBCL tumors while sparing normal blood cells. GAK carries out alignment of the machinery that pulls chromosomes apart during cell division. The team found that exposing DLBCL cells to a GAK inhibitor halted their cell division and promoted programmed cell death (apoptosis), showing their particular dependence on this process to maintain malignant behavior. B-cells are among the most rapidly dividing of all cells in the body, and the DLBCL tumors that derive from the B-Cells grow

aggressively in patients but also are especially dependent on proteins that carry out cell division. Non-malignant blood cells from healthy donors were not substantially affected by GAK inhibition, demonstrating tumor specificity of effects, which should lead to a safe therapeutic window for use in human patients. Other cell-division proteins have been assessed as drug targets in the past in DLBCL and other cancers, and drugs that go against remain under development. The research team's data reveal GAK as a new target with several advantages, including its unique activities during cell division, its highly drug-targetable kinase enzymatic activity, and preliminarily a specific biomarker for tumors likely to be sensitive to its inhibition (dysfunction of a specific tumor suppressor whose loss is a very common driver of malignancy including DLBCL). These findings provide a strong opportunity to develop novel approaches for the treatment of DLBCL and, in the longer term, potentially additional malignancies as well. This application seeks support for these efforts fueled by two specific aims that address the crucial next steps in this process: First, the research team will define in detail the molecular consequences of GAK inhibition in DLBCL experimental systems (Aim 1). These studies will reveal the reasons DLBCL tumors are particularly dependent on GAK for survival and the DLBCL disease subtypes in which GAK inhibition is likely to be most effective. This aim includes also treatment studies in highly accurate animal DLBCL models to preliminarily define the therapeutic window for GAK inhibition in mammalian organisms. Second, a multidisciplinary team of researchers will be leveraged to design and synthesize new GAK-specific inhibitors for further development (Aim 2). Currently, no GAK inhibitor is suitable for testing in patients due to major pharmacologic issues with existing tool compounds, which have fueled their studies to date. The research team already has identified a series of highly promising chemical structures that are predicted by computer modeling to be potent GAK inhibitors and highly suitable for further optimization for eventual use in patients. In particular, the team has identified a strong possibility to generate irreversible inhibitors of GAK through permanent (covalent) binding to the target, a strategy that has yielded some of the most potent and effective drugs against cancer and other diseases in the past. This wholly novel target-validation and drug-development effort combines multidisciplinary expertise into an exciting opportunity to bring about better clinical outcomes for cancer patients in Florida.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

14. **Grant #:** 22B14 The Role of WDR43 in Er-negative Breast Cancer

Principal Investigator: Alvaro Monteiro, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: The long-term goal is to understand the complex interaction between environment and genetics driving breast cancer (BC) risk, with a focus on sub-types prevalent in minority and vulnerable populations. The present application, which is a first step towards this goal, will test the overarching hypothesis that the WD Repeat Domain 43 (WDR43) gene plays a significant role in estrogen receptor (ER)-negative BC by modifying its differentiation program. WDR43 has been unequivocally associated with ER-negative BC risk through powerful epidemiological studies.

The research team proposes the following aims:
1. To determine the landscape of protein-protein interactions of the WDR43 protein. The team will explore the function of the WDR43 protein in an unbiased fashion to determine the landscape of protein interactions in human mammary gland epithelial cells (HMEC) two different approaches: tandem-affinity purification followed by mass spectrometry (TAP-MS) and Yeast two-hybrid (Y2H) screens. Essentially these methods will identify proteins that interact with WDR43 and provide a clue for its function through the analysis of its interacting proteins.

2. To determine the role of WDR43 expression on ER-negative mammary gland cells. The team hypothesizes that modulation of expression of WDR43 will affect the biology of mammary gland cells. The research team will artificially modulate the expression (increase and decrease) in human cells and determine the behavior of cells with changed expression of WDR43. This aim is designed to test the viability of WDR43 as a potential therapeutic target.

Breast cancer is the number one cancer in Florida with an estimated 20,160 new cases to be diagnosed in 2021. The results from the proposed research will impact specifically on a special subtype of breast cancer with poor outcomes and prevalent in a vulnerable population (African American women). Estrogen Receptor (ER)-negative BC, which affects women from every ethnicity, has a worse short-term outcome than ER-positive disease. ER-negative disease accounts for 20-30% of all BC cases, and is more common in premenopausal women and women of African ancestry. More than half of ER-negative BC are known as triple negative breast cancers (TNBC) because the tumors lack the ER, the progesterone receptor, and amplification of Her2/Neu. These tumors are not responsive to routine endocrine therapy or HER2-targeted therapies such as Trastuzumab and Lapatinib. New modalities of therapy are needed to improve outcomes of women with TNBC 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.

William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program
Appendix B
Fiscal Year 2021-2022 Active Grants
Funded Fiscal Year 2020-2021

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
21B01	H. Lee Moffitt Cancer Center and Research Institute	Kenneth Y. Tsai, MD, PhD	$530,900.00	4/30/22	No	No	No
21B02	H. Lee Moffitt Cancer Center and Research Institute	Brian Czwmiecki, MD, PhD	$1,327,721.00	4/30/26	No	No	Yes
21B03	University of Miami	Thomas Malek, PhD	$530,880.00	4/30/24	No	Yes	No
21B04	H. Lee Moffitt Cancer Center and Research Institute	Florian Karreth, PhD	$530,880.00	4/30/24	No	No	No
21B05	University of Florida	Andrew Judge, PhD	$530,840.00	4/30/24	No	No	No
21B06	H. Lee Moffitt Cancer Center and Research Institute	Gina DeNicola, PhD	$530,880.00	4/30/24	No	No	No
21B07	University of South Florida	Rex M. Philpot, PhD	$528,130.00	3/31/24	No	No	No
21B08	Relinquished						
21B09	H. Lee Moffitt Cancer Center and Research Institute	Kathleen M. Egan, ScD	$1,327,120.00	4/30/24	No	No	No
21B10	University of Miami	Noula Shembade, PhD	$530,470.00	4/30/24	No	No	No
21B11	Florida State University	Jerome Irianto, PhD	$265,440.00	3/31/24	No	Yes	No
21B12	H. Lee Moffitt Cancer Center and Research Institute	Matthew Schabath, PhD	$1,327,180.00	4/30/26	No	No	No
21B13	University of Florida	Zhijian Qian, PhD	$530,880.00	4/30/24	No	No	No

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

Principal Investigator: Kenneth Y. Tsai, MD, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

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 NRAS (melanoma oncogene). This subset of melanoma is generally less responsive to immunotherapy and has no available targeted therapy. Approaches to dampen extracellular signal-regulated kinase (ERK) signaling downstream of mutant NRAS have failed to yield meaningful clinical responses in melanoma. Yet stimulating this pathway has not been explored. Interestingly, many BRAF inhibitors (BRAFi) paradoxically activate ERK signaling in RAS-mutant cells. It occurred to the researchers that inducing paradoxical ERK activation in established RAS-mutant cancers, might elevate ERK signaling enough to trigger oncogene-induced senescence. The reserarchers' data show that RAS-mutant cancer cell lines of diverse lineages arrest when exposed 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.

Progress to date establishes that these effects involve certain proteins which appear to be secreted by cancer cells treated with BRAFi. Indeed, just transferring the media that the treated cells have been in is enough to stop proliferation of previous unexposed cells. The researchers have also identified what happens to immune cells around tumors which have been treated with the drug. These clearly implicate T-cells and myeloid cells and give several avenues to further enhance how these drugs can improve responses to immunotherapy.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Brian Czwmiecki, MD, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: This study involves a clinical trial in patients with metastatic human epidermal growth factor receptor 2 (HER2) positive breast cancer. All regulatory approvals have been obtained and have accrued the first two of anticipated 12 patients. The first patient is on active dendritic cell (DC1)vaccine delivery with Pepinemab and is about to have peripheral blood harvested for CD4 Th1 expansion. The second patient began therapy in early August. No significant toxicities have been experienced at this point. It is too early to determine any responses at this point. The researchers are optimistic the accruals on this study will be completed during the funding period. The researchers also have pre-clinical study assessing the the mechanism of action of CD4Th1 adoptive therapy in mouse metastatic mammary carcinoma. The team has found that two doses of alternating CD4Th1 expanded in IL15 and IL7 caused greater response of mammary carcinoma. The researchers are going to be delivering some of the CD4Th1 with the HER2 pulsed DC1 in the tumor environment because the team has demonstrated that CD4 Th1 are responsible for licensing the DC1 that causes the tumors to survive for long periods of time and also migrate to other sites of tumor. These specially activated CD141+ DC1 have not be administered to patient with breast cancer until it has been done it in an earlier breast cancer neoadjuvant study the researchers are conducting.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: A patent has been filed for using CD4Th1 expanded in either IL7 or IL15 for adoptive transfer to patients with HER2 metastatic breast cancer.

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

Principal Investigator: Thomas Malek, PhD

Organization: University of Miami

Abstract: The overall goal of the project is to assess a novel IL-2 analog, IL-2/CD25, to enhance tumor immunity and determine its mechanism of action. Past work showed that IL-2/CD25 plus soluble peptide vaccines supported anti-tumor responses in pre-clinical studies in model systems using monoclonal CD4+ and CD8+ tumor-reactive T cells and in a more physiological polyclonal setting. During the last year, studies were undertaken to refine the monoclonal T cell model to study the contribution a CD4+ tumor-specific T cells in anti-tumor responses. The first step defined the minimal number of monoclonal T cells that lead to detectable IL-2/CD25-dependent expansion. This step is aimed at establishing a model that would approximate a physiological setting with a low frequency of tumor-reactive T cells as this is necessary to study the interplay between tumor-reactive CD4+ and CD8+ T cells. One unexpected finding is that the CD4+ T cells do not enhance the CD8+ T cells response, which is a critical cell in mediating tumor rejection. Since peptide vaccines are not robust, other vaccine formats will be tested. Initially, a dendritic cell-based tumor peptide vaccine was tested, but these were shown not to be more effective than using soluble peptides. Other experiments examined whether more frequent administration of IL-2/CD25 might increase endogenous tumor-reactive T cells to bypass the vaccine. Under these conditions, IL-2/CD25 supported excellent anti-tumor responses in pre-clinical studies for tumors that were immunogenic. This approach was less effective for non-immunogenic tumors, where a vaccine approach might still be beneficial. Mechanistic studies for immunogenic tumors revealed that IL-2/CD25 monotherapy supported a tumor-microenvironment with enhanced number and function of tumor-reactive T cells while limiting the number of regulatory T cells, which may suppress the anti-tumor response. The potential relevance of these findings is that the anti-tumor activity supported by IL-2/CD25 may eventually become a new treatment for immunogenic cancers, such as melanoma or lung cancer, for patients that fail other therapies. As the IL-2/CD25 fusion protein has been licensed to a large pharmaceutical company, these and other discoveries from this project may impact the lives of Florida resident with cancer.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Hernandez, R., Põder, J., LaPorte, K.M., Malek, T.R. Engineering IL-2 for immunotherapy of autoimmunity and cancer. Nat. Rev. Immunol. doi: 10.1038/s41577-022-00680-w. 2022. Online ahead of print.

Patents: None at the time of reporting.

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

Principal Investigator: Florian Karreth, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: The American Cancer Society estimated >100,000 new melanoma cases and ~7,000 deaths in the US in 2020. Approximately 8,750 Floridians develop melanoma and 430 patients will succumb to this malignancy every year. While the majority of cases of localized melanoma are cured by surgical removal of the tumor, the five-year survival rate of metastatic melanoma is only 20%. The incidence of melanoma has tripled over the last 30 years. While the development of immunotherapies has had a significant impact on clinical outcomes, overall these statistics

demonstrate that metastatic melanoma remains difficult to treat and therapies for metastatic melanoma require significant improvement.

The Phosphatase and Tensin Homalog deleted on Chromosome 10 (PTEN) has tumor suppressive activity and its expression is reduced in up to 60% of melanoma cases. While it is well established that PTEN opposes the activation of the PI3K/AKT pathway, the inhibition of AKT in melanoma has yielded disappointing preclinical and clinical results. A potential explanation is that PTEN antagonizes numerous pathways in addition to AKT via its lipid and protein phosphatase activities, and loss of PTEN leads to activation of all of these pathways. Indeed, using an innovative melanoma mouse model the research team observed that restoration of PTEN (and thus all of its functions) in PTEN-deficient melanomas completely halted tumor growth in vivo.

The researchers are investigating the relative contribution of the PTEN lipid and protein phosphatase activities to melanoma suppression. Moreover, the team is defining the pathways that cooperatively drive melanoma formation and survival upon PTEN inactivation. Upon the successful completion of the project, the researchers will have assessed the tumor cell-intrinsic effects of inhibiting PTEN-loss activated pathways and tested if inhibition of these pathways cooperates with immune checkpoint blockade.

Over the last year, the AKT pathway has been validated to not be critical for PTEN loss-mediated melanoma progression using pharmacological and genetic approaches. Using both in vitro and mouse modeling approaches, it has been further shown that it is PTEN's lipid phosphatase activity but not its protein phosphatase activity that predominantly suppresses melanoma development. The research team has performed phosphoproteomics to identify putative PTEN effector pathways that are PIP3-dependent but AKT-independent. Over the next year, the plan is to examine these pathways and test their contribution to PTEN loss-mediated melanoma formation. The team will also determine if these pathways regulate the interaction of melanoma cells with the tumor immune microenvironment. These planned experiments will help identify and assess potential strategies to therapeutically target 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 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 diminishes physical function and quality of life. It is also 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. Ursolic acid is a natural compound derived from several edible herbs and fruits, including apples, that has been shown to reduce muscle atrophy in various rodent models but, to the researchers' knowledge, has never been tested as a countermeasure to cancer-induced muscle wasting. Thus, in proposed studies the research team aimed to conduct a pre-clinical trial of ursolic acid in multiple models of cancer cachexia. In the past year the team have tested the ability of ursolic acid to counter muscle and fat wasting in 5 pre-clinical models using mouse or human colon cancer cells, mouse or human pancreatic cancer cells, and mouse lung cancer cells. The research team has found, in each of these models, that the cancer-induced muscle wasting and fat wasting in mice consuming a control diet is inhibited in mice with tumors consuming a diet supplemented with ursolic acid. This protection is independent of any effect on tumor growth. Interestingly, the team found that ursolic acid consistently showed the greatest protection against cancer-induced soleus muscle wasting in each model, and studies by others have shown that the mouse soleus muscle bears the greatest resemblance to human skeletal muscles. These findings are encouraging in both the consistent protection that ursolic acid provides against muscle and fat wasting induced by different tumors, and in the potential to translate these findings to humans. In the next phase of experiments, the team will treat tumor bearing mice with chemotherapy and ursolic acid, to determine whether the protection against muscle and fat wasting is maintained. Such studies will help inform when might be the best time to intervene with ursolic acid.

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 Pyridine Nucleotides: Missing Link Between Aging and Lung Cancer

Principal Investigator: Gina DeNicola, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

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 mutations that drive cancer, many lung cancer patients do not respond to these treatments or relapse following initial response. Therefore, a more comprehensive understanding of the molecular circuits that underpin lung tumor formation is needed to enable the development of better 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. The research program is aimed at starting to fill this knowledge gap by understanding how age-induced changes in pyridine nucleotide metabolism contributes to tumorigenesis both by exerting effects in lung cancer cells as well as in associated macrophages. The research team's results to date suggest that quinolinic acid, a precursor for pyridine nucleotides synthesis increased in circulation by the aging process, may promote contribute to lung tumorigenesis independently of its function as a precursor for pyridine nucleotide synthesis. Moreover, the team has also uncovered the rate-limiting enzyme in quinolinic acid’s metabolic pathway to be a

metabolic vulnerability of lung cancer cells whose inhibition causes cell death, putting forward the idea that inhibitors of this enzyme might be good therapeutic targets to effectively treat lung cancer. On the other hand, the research program puts forward the idea that pyridine nucleotide metabolism also affects lung tumorigenesis by causing a shift towards more inflammatory macrophage phenotypes. The research team is currently working towards testing this possibility and evaluate whether increasing pyridine nucleotides in the macrophage compartment suppresses lung-cancer associated macrophage inflammation and thereby have beneficial effects for lung cancer patients.

Follow on Funding: None at the time of reporting.

Collaborations: Translational Research Institute, AdventHealth, Orlando, Florida, Dr. Stephen Gardell. Dr. Gardell is a Co-PI on this project and is performing the mass spectrometry and analyses for the measurement of NAD+ and related metabolites in cells and tissues.

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 M. Philpot, PhD

Organization: University of South Florida

Abstract: In the first full year of this project the lab has established the breeding colony of breast tumor bearing mice to model breast cancer, monitored tumor emergence and progression in animals, and assessed cognitive function in chemotherapy exposed animals treatment for chemobrain. During this time measurements of estrus cycling, collection of blood to isolate serum samples and collection of tissue from critical brain regions was performed by research personnel. These samples have been stored at -80C as behavioral assessment of additional groups of animals continues and will be assayed once sufficient sample numbers from all groups have been collected. During this time the research team has also established a radioassay of the quantification of choline acetyltransferase and established and tested dosing concentrations of the experimental drugs PNU-282987 and RJR-2403, verifying that the drugs are safe for daily administration over the course of the studies.

To date the researchers have determined that chemotherapy reduces cognitive function in tumor bearing mice but not non-tumor bearing mice. This indicates that tumors produce a vulnerability to chemobrain, requiring study using tumor bearing animal models. Importantly, the team has determined that neither having tumors nor chemotherapy reduces the amount of choline acetyltransferase, an enzyme important for the production of the neurotransmitter acetylcholine, in brain regions that are important for learning and memory. Because acetylcholine is important for learning and memory it is critical that this enzyme be available for normal cognitive functioning. Assays of samples collected from tumor bearing mice unexposed and exposed to chemotherapy will determine whether the activity, rather than the amount, of this enzyme is reduced by cancer or chemotherapy and in this way leads to a impairment of neurotransmitter and brain function which manifests as chemobrain. Additionally, these measurements of alterations in enzyme activity will be related to measures of cognitive function

from animal behavior once sufficient numbers of animals have been run to perform this type of analysis.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Kathleen M. Egan, ScD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: The goal of this project is to build infrastructure for breast cancer prevention-focused research in Florida that addresses racial/ethnic disparities in breast cancer incidence and outcomes. Study participants include postmenopausal women with no history of cancer (other than non-melanoma skin) that are identified in mammography clinics affiliated with the University of Florida at Jacksonville, Moffitt Cancer Center in Tampa (MCC), and the University of Miami. These clinics draw from catchment areas of the three study centers and offer a broad diversity of women on race/ethnicity and socioeconomic status (SES). In the first project year, surveys were developed and refined, procedures were implemented for collection and shipment of samples (urine; DNA; and stool), and a database management system was put in place for electronic consent of women, collection of surveys (basic risk factor; residential history; diet history questionnaire) in clinics or at home, and management of data across three study centers. Both English and Spanish-speaking women are eligible and all patient-facing study materials are available in both languages. All women provide a spot urine sample and a saliva sample at the time of recruitment. Stool samples are collected at home. Survey data and mammogram images are also collected from study women. Data and biospecimens are shipped to and stored centrally at the coordinating center at MCC. Throughout development of procedures and surveys, input was sought from the Community Advisory Panel and members of the Scientific Advisory Board. After delays related to Covid, recruitment was launched in clinics in Spring 2022. A total of 96 women were enrolled to date and have completed study procedures with an average of approximately four to five women recruited per week across the three study centers. Resources developed in the project will provide an invaluable foundation for a wide range of studies on the environmental, social, and behavioral determinants of breast cancer risk that can be targeted for prevention.

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 herpesvirus (KSHV), and Human T-lymphotropic virus type 1 (HTLV-1) chronically activate NF-κB, a transcription factor that induces inflammation essential for the development of Adult T-cell leukemia, Burkitt lymphoma, diffuse large B-cell lymphoma, primary effusion lymphoma, and Multicentric Castleman disease. Current treatments for viral-mediated chronic NF-κB-induced inflammation are ineffective. Therefore, this application is focused on determining host molecules and mechanisms that regulate NF-κB activation in EBV, KSHV, and HTLV-1 infected cells.

Cells have evolved negative feedback mechanisms to suppress NF-κB signaling and inflammation, which viral oncogenes disrupt, but the mechanisms are unknown. The researchers recently identified T-Cell Lymphoma Invasion And Metastasis 1 (TIAM1) as an essential player required for HTLV-1, EBV, and KSHV to activate NF-κB and inflammation. TIAM1 also inhibits pro-inflammatory cytokine-induced NF-κB activation. These preliminary studies suggest that phosphorylation and ubiquitination convert TIAM1 from an inhibitor to an activator. Based on these novel findings, the Specific Aims are to: 1: Determine the mechanisms of TIAM1 regulation by HTLV-1, EBV and KSHV oncogenes, 2: Determine the mechanistic roles of TIAM1 phosphorylation and K63-linked ubiquitination in the activation of NF-κB in virally transformed cells, and 3: Determine the mechanisms of NF-κB regulation by the CADM1/TIAM1 complex in the context of viral oncogenes and TNFα and IL-1β stimulation.

In the first year, the research team mostly completed Aim 1. Cell Adhesive Molecule 1 (CADM1) is required for TIAM1 phosphorylation and K63-linked polyubiquitination, chronic NF-κB activation, and inflammation induction in cells expressing HTLV-1, EBV, and KSHV oncogenes. The team found four phosphorylation sites in TIAM1 after TNF stimulation, and the HTLV-1 oncogene Tax inhibits this, resulting in two additional amino acid phosphorylations. Thus, TIAM1 phosphorylation mutant(s) stable cell lines were created to investigate its role in the regulation of NF-κB activation.

The findings from this application will pave the way for therapeutic intervention in malignancies caused by multiple oncogenic viruses.

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 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 metastasis-associated 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, the research staff 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, the research staff hypothesize that metastasis of breast cancer cells and change in microenvironment stiffness contribute to the mutations that are essential to metastatic progression. The research staff 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 stiffnesses and their genomes will be quantified and compared to corresponding patient-derived genomic data. The causal effect of the mutations identified from this research will be validated in animal model of breast cancer.

The research staff will dissect the progression of breast cancer metastasis from the novel perspective of mechanobiology and genomics. The proposed research will expand the fundamental knowledge and mechanistic understanding of metastasis associated mutations in breast cancer. Importantly, the 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.
During this reporting period the research staff have made advancements in all of the Aims proposed in this study, specifically:

Aim 1: The researchers have securely downloaded the genomic data of metastatic luminal A breast cancer from the study by Yates, LR et al. (2017, Cancer Cell). From this genomic data, the team has identified the chromosome copy number changes that are associated with metastasis. Researchers have also successfully established the culture of breast cancer organoids in the laboratory and are ready to proceed to perform the cell migration experiments proposed in Aim 1.

Aim 2: The researchers have established and fully characterized the polyethylene glycol hydrogel culture system in the laboratory. Researchers are ready to introduce the breast cancer organoids to this culture system to perform the experiments proposed in Aim 2, which is to study the impact of microenvironment stiffness.

Aim 3: The researchers have established the CRISPR activation/interference system that was proposed in Aim 3, this will enable the team to perform genetic modulation of the breast cancer

organoids. The CRISPR system will be introduced to the breast cancer organoid. The researchers have acquired the DEA license to purchase reagents required for the animal works proposed in Aim 3. The required trainings have started to establish the animal model in the laboratory.

Follow on Funding: None at the time of reporting.

Collaborations: Research staff have initiated a regular monthly meeting with Dr. Xian Fan's laboratory, to discuss the bioinformatics analysis in this project. These meetings are attended by all of the personnel involved in this project.

Journals: The continuous collaboration with Dr. Xian Fan has resulted in a manuscript entitled "Genomic heterogeneity in pancreatic cancer organoids and its stability with culture" by Usman O, Zhang L, Xie G, Kocher HM, Hwang C-I, Wang YJ, Fan X, and Irianto J. The manuscript is now available in the preprint platform BioRxiv (https://doi.org/10.1101/2022.07.03.498602).

Patents: None at the time of reporting.

11. **Grant #:** 21B12 Non-Invasive Radiomic Biomarkers to Predict Treatment Response for Immunotherapy of Lung Cancer.

Principal Investigator: Matthew Schabath, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: The research team has conducted formative studies demonstrating radiomic features (i.e., imaging data calculated from medical images such at CAT scans) predict lung cancer immunotherapy (IO) response. Building on this work, in this multi-institutional study funded by the Florida Biomedical Program, the research team will develop and validate radiomic signatures to predict lung cancer IO treatment response. The short-term translational implications of this work are that with sufficient sample size and thorough testing and validation, the team can generate critical data that can be leverage towards future clinical trials to determine the clinical utility of these models. The long-term translational implications are that radiomic models can provide decision support information that can be provided back to the radiologist and/or oncologist and incorporated into clinical practice to inform rapid decision making for patient treatment.

For the first year of this award, there have been no substantial challenges, delays, and issues. The research team spent the first year identifying the patients for the proposed research and collecting their images and patient data. Specifically, the research teams at the Moffitt Cancer Center and University of Florida have been identifying and curating computed tomography (CT) images and clinical data on lung cancer patients treated with immunotherapy and patients treated with tyrosine kinase inhibitors (TKIs). To date, the researchers have curated images (baseline images +/- pre-baseline images +/- PETCT images) from 962 patients treated with immunotherapy and 250 patient treated with TKI (baseline images +/- pre-baseline images +/- PETCT images). Since data collection efforts are still underway, no results on the planned analyses from these patient cohorts have been generated. Once the data curation efforts are completed, this will be the largest resource of its kind in the world.

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: This study aims to get a better understanding of the role and underlying mechanism of ALKBH5 in Acute myeloid leukemia (AML), which is one of the most common types of leukemia. 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. The 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. The research team found that ALKBH5 is upregulated in human AML and that its overexpression is associated with poor prognosis in AML patients. The functional studies showed that ALKBH5 plays a critical role as an m6A demethylase in the development and maintenance of AML caused by an MLL-rearrangement (MLL-AF9) and that it is essential for leukemia stem cell/leukemia-initiating cell (LSC/LIC) self-renewal.
During this period, the research team has made some significant progress on this project. The researchers have generated a mouse transplantation model expressing both AML1-ETO-9a and N-RAS mutant and demonstrated that the mice developed AML diseases, four months post-transplantation. In addition, the team has initiated the development of a new conditional ALKBH5 transgenic model [Tg (LSL-ALKBH5)] using the new TARGATTTM system (site-directed gene integration) in the C57B6 background strain. In this mouse model, the expression of the human ALKBH5 gene can be activated. The team monitored the mice with the expression of the human ALKBH5 gene for several months and found that the mice developed abnormal hematopoiesis. To further understand the function of ALKBH5 in leukemogenesis, the research team searched for the binding proteins of ALKBH5 in leukemia cells and found that RBM33 is a binding partner of ALKBH5. The researchers hypothesize that ALKBH5 plays a critical role in subsets of AML by regulating protein expression through its demethylase activity. For this reason, the research team believes that ALKBH5 may be a common therapeutic target for subsets of AML with MLL-rearrangements and AML-ETO.

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.

William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program

Appendix C

Fiscal Year 2021-2022 Active Grants

Funded Fiscal Year 2019-2020

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
20B01	All Children's Research Institute	Masanobu Komatsu, PhD	$636,611.00	4/30/23	Yes	Yes	No
20B03	H. Lee Moffitt Cancer Center and Research Institute	Shain Kenneth, MD, PhD	$636,610.00	5/31/23	Yes	No	No
20B04	H. Lee Moffitt Cancer Center and Research Institute	Paulo C. Rodriguez, PhD	$636,610.00	5/31/23	No	Yes	No
20B06	H. Lee Moffitt Cancer Center and Research Institute	Andriy Marusyk, PhD	$636,610.00	6/30/23	Yes	No	No
20B07	RELINQUISHED						
20B08	H. Lee Moffitt Cancer Center and Research Institute	John M. Koomen, PhD	$253,555.00	5/21/23	No	No	No
20B10	H. Lee Moffitt Cancer Center and Research Institute	Nicholas J. Lawrence, PhD	$636,610.00	11/30/22	No	Yes	Yes
20B11	University of Florida	Elias J. Sayour, MD, PhD	$636,610.00	5/31/23	No	No	No
20B12	University of Miami	Sabita Roy; PhD	$636,610.00	5/31/23	No	No	No
20B13	University of Miami	Jaime R. Merchan, MD, MMSc	$636,610.00	10/31/22	No	No	No
20B14	University of Miami	Marzenna Blonska, PhD	$636,610.00	5/31/23	No	No	No
20B15	University of Miami	Lluis Morey, PhD	$636,610.00	5/31/23	Yes	Yes	No
20B16	University of Miami	Paulo S. Pinheiro, PhD	$750,000.00	5/31/23	No	Yes	No
20B17	H. Lee Moffitt Cancer Center and Research Institute	Chen Jiandong, PhD	$636,610.00	5/30/23	No	Yes	No

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

Principal Investigator: Masanobu Komatsu, PhD

Organization: All Children's Research Institute

Abstract: In this project, researchers set out to investigate the role of tumor vasculature (blood vessel network of tumors) in shaping the immune environment in the tumors. Cancer patients can develop immune response against their tumors while other patients don't. The stronger the patient's immune reaction to the tumor is, the better prognosis the patient has. Patients with anti-tumor immunity respond to chemotherapy, immunotherapy, and other cancer therapies. Accumulating evidence suggests that tumor vasculature plays a critical role in regulating patients' immune response tumors because the circulating immune cells are recruited to the tumors via blood vessels. The research team therefore investigated the key molecular differences between the blood vessels of immune responsive tumors and non-responsive tumors.

This study identified 76 differentially regulated genes in these two types of tumor blood vessels in clinical specimens of breast cancer. Of particular interest were a tetraspanin gene TSPN7 and a homeobox gene MEOX2. The high transcript count of these two genes predicted survival of breast cancer patients. In this study, researchers also identified the existence of PNAd-negative high endothelial venule-like blood vessels in the immune hot areas of breast cancer. These blood vessels are TSPAN7+ and surrounded by extensive clustering of lymphocytes. Close examinations of histological sections with high power microscopy suggest that lymphocytes extravasate through these vessels, suggesting a role for non-PNAd blood vessels in developing anti-tumor immunity. The molecular signature of tumor blood vessels identified

here may be useful for guiding immunotherapies and provide a new direction for investigating tumor-associated high endothelial venules and their clinical significance. The manuscript to report these findings was recently published by Cancer Immunology Research, and this article was highlighted in “In the Spotlight” of the same issue of the journal recognizing the significance of the finding for the immuno-oncology field. This study was also featured online by the Johns Hopkins All Children’s Hospital News Letter for general public. The molecular signature of high endothelial venules identified in this study may be useful for guiding immunotherapies and provide a new direction for investigating tumor-associated blood vessels and their clinical significance.

By studying tumor blood vessels of immune reactive and non-reactive tumors of many cancer patients, researchers were able to identify two important immune-stimulating factors (agonists) that facilitate creation of immune reactive tumor environment. In the second part of the year, the research team mainly focused on investigating the effect of these agonists on the development of immune response and the consequential tumor cell killing by immune cells using mouse tumor models.

The study demonstrated that the two agonist combination halts the growth of subcutaneous pancreatic tumors and orthotopic mammary tumors. This therapeutic effect did not appear to be dependent on the presence of tertiary lymphoid structures (TLS), dense clusters of tumor-reactive immune cells, in the tumors. However, the presence of TLS correlated the prevention of tumor recurrence and long-term survival, indicating that TLS formation in the primary tumors boosts

Follow on Funding: NIH/National Cancer Institute, Masanobu Komatsu, Ph.D., 4/1/2022 - 3/30/2027, Requested: $2,046,875, R01, Funded: 1,872,890

Collaborations: None at the time of reporting.

Journals: Sawada J, Hiraoka N, Qi R, Jiang L, Fournier-Goss AE, Yoshida M, Kawashima H, Komatsu M. Molecular signature of tumor-associated high endothelial venules that can predict breast cancer survival. Cancer Immonology Research. 2022; 10(4):468-481. PMID: 35201

Patents: None at the time of reporting.

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

Principal Investigator: Shain Kenneth, MD, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: The previously stated goal is to prevent XPO1 binding with novel NES inhibitors (NESi) and thus keep TOP2A in the nucleus, with the expected outcome of sensitizing Multiple Myeloma (MM) cells to PLD and the development of a new class of drugs for MM and other malignancies. The researchers proposed the following aim to carry-out this goal(s).
Aim 1: Synthesize new NESi that block TOP2A-XP01 binding and characterize their activity.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 the ZINC repository in silico. Aim 3: Optimize and define the activity of the NESi discovered in Aims 1 and 2.

1-Within this funding period, the researchers have continued to determine the relevance of the initial findings in multiple cell line models (naïve and drug resistant) as well as patient specimens, to begin testing newly identified molecules from the screens in the same in vitro, in vivo and ex vivo models, and finalize the second NES-based manuscript (Targeting the Nuclear Export Signal of Topoisomerase IIα in Multiple Myeloma; Target Journal Molecular Cancer Therapeutics).

2-To examine the biological pathways associated with TOP2A inhibition, the research team has translated the in vitro and in vivo findings in multiple myeloma patients. Ex vivo analysis of Dox/NSC9138 activity has been assessed in 23 patient specimens. Importantly, while in all samples the additive activity was greater than either drug alone, synergy was observed in a minority 33% suggesting that although the concept of enhanced TOP2A activity in the presence of NESi does occur, it remains cell (or patient) dependent- e.g. not all patients (cells) will be sensitive to the combination. The research team has collected and are carrying WES and RNAseq on these patient specimens (funding outside context of award) with goal of better understanding the MOA of this combination and/or mechanisms of resistance.

3-The research team continues to identify and prepare additional TOP2 NESi for the in vitro screening platform

4-As reviewed in the last quarterly update, the researchers are translating these same techniques to additional NES containing proteins. With the collaborative team at UF, the researchers identified the NES of MDM2 & MDM4 using AlphaFold. The NESi- 188 ser to 200 cys interaction predicted by AlphaFold MDM2 residues 25-27, ETL and 110-114 VNQQE. Interestingly, this NES t has better predicted binding than the TOP2 NES. Working with MDM2 signaling experts at Moffttt, the research team is establishing screening tools (MCF7 breast cancer p53-luc reporter systems- as cell viability may not be the appropriate end point for targeting MDM2) to begin to screen compounds identified in the screen.

Impact to Floridians: Within the context of this proposal the researchers have used the plasma cell malignancy Multiple Myeloma as a cancer model system to identify a novel -protein specific- drug development system. To mitagate signaling via blocking the normal nuclear export of specific proteins and sensitize to current therapetics. The success of this project has a much larger breadth with broad applicability to all cancers treated with topoisomerase II inhibitors (breast cancer, sacrcoma, lymphoma, etc). One of the novel aspects of this collaboration is that the same process may be successful in targeting one of the most common pathways in cancer- the TP53/MDM2 master regulator of cell fate. As such, the researchers anticipate this evoluationary step has even a greater potential to improve outcomes for Floridians with cancer (and beyond).

Follow on Funding: Kenneth H Shain & Rachid Baz, 08/01/2021-07/31/2026, Philanthropic Funding, Funded: $10,000,000

Collaborations: The collaboration for this grant remains well and intact. As described in the previous section, the collaboration has manifest into additional research endeavors utilizing the overlapping drug development pathways. Further, within the context of this grant the collaborating team with intra-Moffitt as well as the researchers at the University of Florida (Dr. David Ostrov use the crystal structure of TOP2A and MDM2 to select additional NESi as well as other drug targets by screening large chemical libraries in the ZINC and Moffitt Life chemicals repository in silico.

As this is a group of investigators across the state of Florida, the coordination of projects and effort is critical. With continued improvement in the COVID-19 pandemic setting researchers anticipate increased in person meetings this quarter. Regardless, researchers will continue to have meetings via zoom monthly with Dr. Ostrov (UF investigator), Dr. Lawrence, Dr. Kenneth, and their respective teams. This has facilitated a great deal of collaborative effort for carrying out the goals of this important FL DOH grant as well as the construction of the first manuscript from this proposal. If and/or when it becomes appropriate in the setting of COVID researchers will also carry-out in-person half day meetings at least twice yearly alternating between Moffitt (Tampa) and UF (Gainesville).

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Paulo C. Rodriguez, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: Therapeutic actions of tumor-specific T cells in solid malignancies remain ineffective. The researchers aimed to create new strategies that make T cells refractory to tumors 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.

Period 1 (From: 06-01-2021 Through: 09-30-2021). During this cycle, the research team evaluated the killing efficacy of human melanoma specific NY-ESO-TCR T cells developed in conditions that promote Notch signaling against target tumor cells in vitro. Results show higher anti-tumor effects and expression of cytotoxic mediators in NY-ESO-TCR T cells co-cultured with DLL4.v3 expressing K32A2 (High Notch conditions), compared to counterparts exposed to Mock or WT-DLL4.

Period 2 (From: 10-01-2021 Through: 12-31-2021). The research team continued expanding on the phenotype of engineered T cells cultured in high Notch conditions. It was found that NY-ESO-TCR T cells having higher Notch signaling exhibited significant changes in several cytokines. Another key component of the studies assessed the effect of the expression of Notch1 intracellular active domain (N1IC) in the activity of CD8+ T cells in murine models, which showed that Notch1 signaling promotes higher effector activity, and elevated glycolysis and mitochondrial respiration in activated CD8+ T cells.

Period 3 (From: 01-01-2022 Through: 03-31-2022). The research team started establishing the conditions for the therapeutic evaluation of Notch-active T cells in mice bearing tumors. Also, the team investigated whether elevated Notch signaling renders T cells less susceptible to tumor-linked mitochondrial stress. Results showed that Notch-active CD8+ T cells were able to retain their superiority to produce effector mediators in the presence of a mitochondrial stress settings.

Period 4 (From: 04-01-2022 Through: 06-30-2022). The research team continued testing the therapeutic effect of engineered T cells in cancer-bearing mice. Results show that manufacturing CAR-T cells in settings that promote Notch signaling augments their therapeutic

effectiveness after adoptive transfer into tumor-bearing hosts. Also, new results support the postulate that Notch signaling protects T cells from mitochondrial stress by maintaining the cells in stem cell-like memory stages and potentially through regulation of mitochondrial stress driver LonP1.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Decoding endoplasmic reticulum stress signals in cancer cells and antitumor immunity. Salvagno C, Mandula JK, Rodriguez PC, Cubillos-Ruiz JR. Trends Cancer. 2022 Jul 8: S2405-8033(22)00134-0. DOI: 10.1016/j.trecan.2022.06.006. Online ahead of print. PMID: 35817701.

Tumor-directed dysregulation of erythroid progenitors drives immunosuppressive myeloid cells. Mandula JK, Rodriguez PC. Cancer Cell. 2022 Jun 13;40(6):597-599. DOI: 10.1016/j.ccell.2022.04.017. ePublication 2022 May 19. PMID: 35594864.

Patents: None at the 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 and Research Institute

Abstract: Research project staff has refined and validated the digital pathology platform that has been developed during the previous reported period. Moreover, the pipeline was extended to include incorporation of analyses of immunofluorescent data as well as adding new spatial ecology metrics.

Research project staff has applied the spatial analyses developed with support of this grant to multiple experimental models of acquired therapy resistance towards targeted therapies in lung cancers. These analyses enabled the research staff to quantitatively understand the impact of stromal architecture on the emergence of resistance within intially therapy sensitive tumors.

The research team has applied the spatial analyses pipeline to understand the competitive dynamics between therapy sensitive and therapy naïve subpopulations under selection pressures of targeted therapies in experimental models of targeted therapies in lung cancers. Research staff has optimized the initial version of spatial agent based models intended to capture the impact of stromal architecture on therapy responses and applied this model to evaluate different strategies for interfering with stromal sheltering towards improving therapeutic responses.

Research project staff have refined the digital pathology platform (both segmentation and analyses parts), including incorporation of analyses of immunofluorescent data as well as adding new spatial ecology metrics, resource selection function.Preliminary data generated in this work was used as a basis of multiple research grant proposals. While none has yet been granted, several proposals have received positive response and will be resubmitted.Research

findings produced in this project are serving as a basis of three manuscripts that are currently being prepared.

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

Principal Investigator: John M. Koomen, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: This project stems from the unique ability to evaluate tissues collected from patients who passed away from metastatic lung cancer that had spread to multiple locations across their body. To optimize therapeutic approaches or develop new treatment strategies for patients with advanced lung cancer, the mechanisms underlying differences in response to treatment for lesions scattered throughout the body must be better understood. Collaboration between an analytical chemist and a molecular pathologist has enabled evaluation of total tumor proteomes as well as quantification of a targeted panel of specific biomarkers to examine differences between these metastatic tumors for several patients. During this year, both expression proteomics and targeted assay datasets were acquired for sets of tumor cancer adjacent control tissues from ten non-small cell lung cancer (NSCLC) patients. All technical hurdles were overcome; datasets could successfully be acquired from thin sections of formalin fixed paraffin embedded tissues. In other words, a minimal amount of tumor tissue was expended to get the amounts of protein (25 micrograms per sample) needed for comprehensive proteome analysis (> 10,000 proteins in the dataset). Data analysis and interpretation are ongoing.

The goal of this phase of the project is to examine the similarities and differences between the metastatic sites within the same patient and to compare heterogeneity between tumors in the same patient and across different patients. The impact of the molecular driver of the cancer and the site of metastasis on the tumor proteome will be interrogated. When possible, differences in the proteome will also be related to differences in response to therapy. In addition, collaborations have been developed with other Moffitt investigators to better understand lung cancers driven by anaplastic lymphoma kinase (ALK) fusions and to develop novel targets for immunotherapy in small cell lung cancer, which will pair with the planned analysis of metastatic tumors from 11 patients that died from small cell lung cancer. The experiments will help to derive knowledge from the discovery proteomics experiments for the ALK-driven and SCLC tumors donated by patients. Together, the goal is to leverage these datasets to better understand complex tumor biology and develop strategies that can address the needs of terminal lung cancer patients.

As a part of the targeted proteomics research, biological annotation of the tryptic peptides from the entire human proteome has also been performed to create a resource for the scientific community. Proteomics researchers typically select the best peptide based on its chemical characteristics, but do not have ready access to the biological significance related to that part of

the protein. This information can further assist peptide selection for protein biomarkers by examining its biological relevance and further examining issues with the sequence, like mutation or post-translational modification, that can impact the ability to detect and quantify these peptides. A software tool is being developed to enable the use of the data by other investigators.

Follow on Funding: None at the time of reporting.

Collaborations: This year included collaborations with other Moffitt faculty, including Andriy Marusyk to better understand the proteomes of ALK-driven lung cancers and Brad Perez in Thoracic Oncology to consider how data from this project can inform novel developments.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

6. **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 and Research Institute

Abstract: Myeloproliferative neoplasms (MPNs) are chronic leukemias composed of three main phenotypes including polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF). MPN prevalence estimates that over 300,000 US patients live, equating to 21,000 Floridians with the disease and all of patients with PV having mutations in a protein called Janus kinase 2 (JAK2) and 60% of MF and ET patients having a JAK2 mutation. For this reason, developing new drugs to target JAK2 for treating myeloid cancers is of critical importance to Florida and the US.

The project team is using an interdisciplinary approach towards the development and characterization of new inhibitors of JAK2 and therefore of MPNs using medicinal chemistry, biochemistry and cell and animal models to improve efficacy and effectiveness over current clinical treatments. This reporting period chemistry efforts have been spent in developing structure activity relationships of a new series of potential JAK2 inhibitors based on novel and patentable phenylpyrimidine and phenylpyrrolopyrimidine scaffolds and PROTAC (proteolysis targeting chimera) molecules. These have been characterized to explore JAK2 inhibition and degradation potential in biochemical and cell line assays. Several of these new compounds are highly potent JAK2 inhibitors. Dr. Schonbrunn, and the structural biology team have solved the X-ray cocrystal structures of one of new inhibitors with the JAK2 kinase domain at a resolution of 2.2 Å, respectively. Both inhibitors bind to the enzyme catalytic site and form two H-bonds with amino acid residue leucine-932 of the hinge region of JAK2. The molecular interactions of the new inhibitors, as revealed by the X-ray structures developed in the project, are facilitating the design of new compounds with improved selectivity for JAK2 through molecular modeling. Importantly the crystal structure reveals the binding mode of the new compounds and the positions where the ubiquitin ligase ligands can be attached via a suitable linker in the design of the PROTACs.

Several of the compounds developed as part of the project both potently and simultaneously inhibit JAK2 and bromodomain-containing protein 4 (BRD4) with potencies comparable or better

than current clinical agents. This leads to demonstrated reduction in cell growth of both MPN and multiple myeloma cells. PROTACs based on these inhibitors have been shown to degrade JAK2 or BRD4 in several cell lines. These data are critical for the next step of the project, which will be undertaken in the final funded year, to show compound activity in mouse models of MPNs to provide evidence of the potential clinical impact of the research. A patent application, based on the research outcomes, has been filed this year, "Structural insights into JAK2 inhibition by ruxolitinib, fedratinib, and derivatives thereof," which seeks to provide protection of intellectual property associated with the new monovalent inhibitors and their PROTAC counterparts. This is important in providing the potential licensing partners (which are being sought through the efforts of the Moffitt Innovation Office) exclusivity to progress a compound to the clinic to benefit patients with MPNs.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Karim RM, Bikowitz MJ, Chan A, Zhu JY, Grassie D, Becker A, Berndt N, Gunawan S, Lawrence NJ, and Schönbrunn E, Differential BET bromodomain inhibition by dihydropteridinone and pyrimidodiazepinone kinase inhibitors, J. Med. Chem., 2021, 64(21), 15772-15

Patents: "Structural Insights Into JAK2 Inhibition By Ruxolitinib, Fedratinib, And Derivatives Thereof," filed January 18, 2022, and assigned PCT Serial Number PCT/US2022/012772. Inventors: N. Lawrence, H. Lawrence, E. Schonbrunn and G. Reuther.

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

Principal Investigator: Elias J. Sayour, MD, PhD

Organization: University of Florida

Abstract: The researchers sought assess resistance mechanisms in RNA-NP treated osteosarcoma models. The research shows that long-term survivors (post-RNA-NP treatment) could successfully ward off obstructive sleep apnea (OSA) tumor re-challenge (without additional treatment). While these results suggest profound memory recall, some animals develop tumor outgrowth (after re-challenge) which warrants exploration of resistance mechanisms in non-survivors. Additionally, the research has shown that RNA-NPs can be enriched for tumor antigens or configured with siRNAs to target pertinent regulatory axes (i.e. PD-L1) for induction of enhanced therapeutic activity. Mechanistic insights gleaned from understanding OSA resistance in development of adaptable RNA-NPs will be utilized.

The researchers found that CD70 RNA-NPs increase expression of CD70 in mouse lungs. Based on these observations and observations that OSA may develop antigen loss, the research team prioritized development of bidirectional RNA-NPs that induce simultaneous expression of surface targets while inducing immunologic response.

To antagonize resistance axis in OSA (myeloid cells expressing CD70) bidirectional RNA-NPs (that express CD70- to mitigate against antigen loss, Aim 1) were utilized in conjunction with CD70 CAR T cells to target immunoregulatory cells and tumor cells expressing CD70. In follow-up experiments, it was shown that these RNA-NPs can mediate substantial regression of

established OSA models when combined with CAR T cells that correlates with T cell mobilization out of the peripheral blood. The research has also shown that optimal anti-tumor efficacy requires balance of both innate and adaptive immune response. In a cohort of canines, mobilization of peripheral blood lymphocytes was shown.

Interestingly, regression of OSA tumors associates with T cell mobilization out of peripheral blood and into both tumors and reticuloendothelial (RES) tissues (i.e., lung, liver, spleen, lymph nodes), a phenomenon that correlates with upregulation of monocyte chemoattractant factors and lymphocyte chemoattractants in vaccine-induced type I interferon (IFN). These marked effects were in the absence of conditioning chemo suggesting that mRNA vaccination may replace conditioning therapy (encumbered by reactive myelopoiesis that inhibit CAR T cells) and can be leveraged for multiple administrations to continually promote in vivo trafficking and persistence of T 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.

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

Principal Investigator: Sabita Roy, PhD

Organization: University of Miami

Abstract: Seventy-two percent 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 the senses, causing one 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 opiods. Opiod use has been shown to induce microbial dysbiosis and systemic inflammation. In previous progress reports, the researchers reported the development of a model for metastatic cancer pain. The current progress report determined that antibiotic treatment prior to cancer cell injection rapidly depleted essential commensal bacteria resulting in decrease in alpha diversity. The researchers previously showed that antibiotic treated mice displayed greater pain sensitivity. From these readouts it was concluded that commensal bacteria are essential to protect against cancer associated pain.

Bioinformatics analysis were performed as described. Demultiplexed sequence reads were clustered into amplicon sequence variants (ASVs) with the DADA2 package (version 1.21.0) (1) implemented in R (version 4.0.3) and RStudio (version 1.1.463). The steps of the DADA2 pipeline include error filtering, trimming, learning of error rates, denoising, merging of paired reads, and removal of chimeras. On average, 21,340 sequence reads per stool sample, 26,800 sequence reads per large intestine sample, and 16,684 sequence reads per small intestine samplewere kept after error filtering and other steps. The ASV table generated by DADA2 was imported into the QIIME2 pipeline for diversity analyses and taxonomic assignment. Diversity analyses were performed by using the qiime diversity core-metrics-phylogenetic script with sampling.

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

Principal Investigator: Jaime R. Merchan, MD, MMSc

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 had developed novel measles virus vectors, which are able to target tumor stroma, as well as murine targeted vectors, allowing researchers to characterize their effects in syngeneic, immunocompetent colorectal cancer models, in addition to human CRC models. The objectives are to characterize a novel virus drug combination, using oncolytic measles viral vectors with triptolide (for in vitro experiments) and minnelide (for in vivo experiments). During the 2021-2022 grant period (ending June 30, 2022), the research team has completed aim 1, and have made significant advanced in aim 2. In aim 1, the team completed mechanistic molecular (proteomic) studies that elucidate in part the mechanisms of enhanced viral oncolysis by triptolide in vitro and had confirmed significant modulation by measles viral vectors of tumor stromal interactions, especially targeting tumor associated macrophages. The more significant progress was made in the in vivo studies, where staff confirmed potent in vivo antitumor activity of minnelide in two different human colon cancer models (HT-29 and HCT-116), as well as in murine colon cancer (CT-26). Moreover, it was demonstrated, in human colon cancer, that minnelide enhances in vivo oncolysis and antitumor activity, by improving viral delivery into tumors, and enhancing the antiproliferative and pro-apoptotic effects of measles viral vectors in the HT 29 model. Similar effects were observed in the HCT-116 model, and current experiments are being performed to confirm the augmentation of viral oncolysis by minnelide in this additional model. The research team expects to complete tumor correlative studies to elucidate the in vivo mechanisms of minnelide's augmentation of viral oncolysis. From year two of the project, it was confirmed the in vitro and in vivo contribution of triptolide and minnelide in the efficacy of oncolytic measles virus and gained significant insight into the mechanisms of these effects. Researchers have presented the results of the above studies in international scientific meetings, such as the 2022 AACR annual meeting and the 2022 ASCO annual meeting. On year three of the grant, aim 3 is expected to be completed as proposed (combination of virus, minnelide and anti-PD-1 agents).

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 #:** 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. The researchers' 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 the hypothesis, the researchers created genetically modified 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), the research team proposed to create mouse models of both diseases. Planed observation time was 15 months. The second aim was identifying the genes controlled by FOXC1 in lymphoma and solid tumors. The research team searched the public repositories and collected large data sets from tumor biopsies, including lymphoma, breast cancer, lung cancer, and melanoma. Based on the relative expression of FOXC1, the patients were stratified into two groups: FOXC1-high and FOXC1-low. The results indicate that FOXC1 regulates genes that facilitate cell migration, invasion, adhesion, and interaction with the tumor microenvironment. Next, study has revealed the mechanism of aberrant expression of FOXC1. It was found that high expression of FOXC1 protein correlates with low methylation of the FoxC1 promoter (part of DNA). Changes in DNA methylation affect the gene promoter accessibility for specific activators. In "normal", mature cells, the FoxC1 promoter is highly methylated, and this factor is not expressed. Finally, the research team identified the compounds that suppress the activation of FOXC1 once it is expressed in tumor cells (potential inhibitors of FOXC1). Top candidates have been selected for translational research that will be further tested in the future.
The study also provides a rationale for screening lymphoma patients for elevated FOXC1.

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

Principal Investigator: Lluis Morey, PhD

Organization: University of Miami

Abstract: Significant progress has been made in all three aims. New Polycomb-mediated epigenetic mechanisms in estrogen receptor positive breast cancer have been discovered. The

scope to the studies have been expanded by generating novel models that mimic resistant to current therapies for ER+ breast cancer patients. Briefly, the researchers found a new way that the breast cancer cell uses to keep proliferating aberrantly and therefore generating tumors. Moreover, the research team has now stablished a strong functional link between Polycomb and resistance to current therapies aimed to target estrogen receptor positive breast cancer, and discovered new molecular mechanisms in cells resistant to encocrine theapies. 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 have been recently pubished in the prestigious journal Nucleic Acid Research (NAR) with an Impact factor of 17 (PMID: 34428304). Moreover, the research team has been working on the RING1B paralog RING1A. Preliminary results suggest that breast cancer cells are also addicted to RING1A. The research team, to their knowledge, has performed the first RING1A chromatin immuniprecipitation experiments to deterime the localization of this protein in the genome. Moreover, the team has identidied the proteome of RING1A and RING1B. After multiple attempts of using the auxin-inducible degron system (AID) to acutely deplete RING1B, it was decided to change strategy because of an expected degradation of RING1B before adding auxin in the knock-in cells. Alternative strategy suing the dTAG (The degradation tag) technology is being set up. Analysis is ongoing with the clones after CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) editing. Regarding the translation approch of the grant, it was initially proposed the use of the RING1B inhibitor PRT4165 but the research team recently obtained the first PROTAC (Proteolysis targeting chimeric) to degrade RING1B and confirmed that it degrades RING1B and affects proliferation of ER+ breast cancer cells. A xenograft experiment is being set up. The final year of the award, the researchers will keep working on the proposed plans and plan to publish at least one more article. During the second year of the award, multiple presentations have been given on the work derived from this grant in both national and international meetings.

Follow on Funding: None at the 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, Tong Liu from the lab of Dr. Wang. Department of Computer Science, University of Miami, Dr. Stransky from the lab of Dr. Simone, Department of Biochemistry, Albert Einstein College of medicine, Bronx, NY, Jian Jin, PhD, Mount Sinai Endowed Professor in Therapeutics Discovery and Director of the Mount Sinai Center for Therapeutics Discovery.

Journals: Liliana Garcia-Martinez, Andrew Adams, Yuichiro Nakata, Ho Lam Chan, Toni Celià-Terassa, Daniel Bilbao, Ramiro Verdun and Lluis Morey. "Endocrine resistance and breast cancer plasticity are controlled by CoREST". Nature Structural and Molecular Biology. 2022 (in press). IF: 15.4

Iván Pérez-Nuñez, Catalina Rozalén, Irene Sangrador, Mariona Dalmau, Laura Comerma, Anna Hernández-Prat, David Casadevall, Silvia Menendez, Daniel Dan Liu, Minhong Shen, Irene Rius Ruiz, Raúl Peña, Jose Carlos Montañés, Maria del Mar Albà, Roger R Gomis, Juan Miguel Cejalvo, Sonia Servitja, Diego Marzese, Lluis Morey, Joaquín Arribas, Begoña Bermejo, Yibin Kang, Joan Albanell, Toni Celià-Terrassa. “LCOR mediates interferon-independent tumor immunity and responsiveness to immune checkpoint blockade in triple negative breast cancer”. Nature Cancer. 2022 Mar 17. IF: 23

Yusheng Zhang, Tong Liu, Fenghua Yuan, Liliana Garcia-Martinez, Kyutae D. Lee, Stephanie Stransky, Simone Sidoli, Ramiro E. Verdun, Yanbin Zhang, Zheng Wang, and Lluis Morey. “The

Polycomb protein RING1B enables estrogen-mediated gene expression by promoting enhancer-promoter interaction and R-loop formation". NAR. 2021. Sep 27;49(17):9768- 9782. IF: 17

Patents: None at the time of reporting.

12. **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 project are to identify critical points in disparities in risk (incidence) and survival for two highly fatal cancers (lung and liver) among 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 second year, finally managing to assemble some key data from the DOH dependencies after major delays. For lung cancer, the researchers have been able to assess patterns among never smokers. For liver cancer, distinct patterns by etiology (alcohol, viral hepatitis, 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. In particular, there is a high incidence of hepatoma (main form of liver cancer) associated with Hepatitis C virus (HCV) among the Puerto Rican population and US-born African Americans. These populations will potentially benefit from HCV screening (and subsequent treatment if tested positive) in the asymptomatic adult population. Based on early promising results, two editorials have been published by the principle investigator, Dr. Paulo S Pinheiro, in the high impact factor Journal of the National Cancer Institute (NCI), and he has also presented at various conferences including NCI's Workshop on Cancer Epidemiology in Hispanic Populations, highlighting the need for results/studies such as this one, as the US population becomes more diverse. Additionally, the main manuscript for Aim 1.1, "A novel analysis of Lung Cancer in Never Smokers: distinct population-based patterns by age, sex, and race-ethnicity", has been submitted for publication and the manuscript for Aim 2, "Incidence of etiology-specific Hepatocellular Carcinoma; diverging trends and remarkable heterogeneity by race-ethnicity", is under preparation. Two new abstract presentations pertaining to Aim 1.2, titled "Racial Disparities in Receipt of Curative Surgery for Early Stage Non-Small Cell Lung Cancer in Florida" and "Racial Disparities in Curative Surgery for Black Early Stage Non-Small Cell Lung Cancer Patients in Florida, 2005-2017" were also presented at the ASCO and NAACCR conferences.

Follow on Funding: None at the 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. During March 2021, a minority student in the epidemiology program at FAMU, Ms. Kamaria Jacobs, doctoral candidate, was identified to be part of the team's project. Additionally, Ms. Qinran Liu, doctoral student in epidemiology, at UM has been added to the study team. An

initial introductory meeting was held with the doctoral students in May 2021 and as of July 2021 the students have commenced work on the study. Students will gain valuable experience and mentorship from the Study Investigators and team.

Following the presentation for the 2021 NCI's Cancer Epidemiology in Hispanic Populations Virtual Workshop, Dr. Pinheiro has now been invited to work as a Consultant on Cancer in Hispanics Surveillance Systems with NCI's SEER Program.

Journals: Pinheiro PS. Cancer Mortality in Latino Populations by Birthplace and Generation: A Complex Analysis. Journal of the National Cancer Institute 2022; 10.1093/jnci/djac079.

Pinheiro PS. Cancer Surveillance Opportunities to Meet Prevention and Control Challenges. Journal of the National Cancer Institute 2021; 10.1093/jnci/djab132.

Liu Q, Jacobs KT, Lopes G, Brown CP, Pinheiro PS. Racial disparities in receipt of curative surgery for early-stage non–small cell lung cancer in Florida. Journal of Clinical Oncology 2022; 40(16_suppl): 8539-8539.

Patents: None at the time of reporting.

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

Principal Investigator: Chen Jiandong, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: In the past year, the team's research effort to develop p53 inhibitors for improving the safety of cancer chemotherapy have been directed to several areas: 1) Testing the ability of soluble NSC194598 derivative compound #147 to inhibit p53 DNA binding in cell-free assays and in cell-based assay. The work was mostly successful, providing us with a new p53 DNA binding inhibitor with better solubility and potency. 2) Testing whether #147 inhibits p53-mediated cell death in culture. Positive results were obtained showing the protective effect of #147 after activating p53 using mdm2 inhibitor Nutlin, and activating a temperature sensitive mutant p53 in a lymphoma cell line. However, no protective effect was observed using several chemotherapy drugs. It is unclear whether this was due to suboptimal assay design or a true absence of such activity. 3) Testing the ability of #147 to inhibit p53 activation and protect mice form radiation toxicity. This work was largely successful in demonstrating that #147 improved survival of mice after lethal dose gamma irradiation. 4) Analyzing the binding between p53 and #147 by nuclear magnetic resonance (NMR) and cocrystalization. This work has so far failed to detect binding. The major obstacle encountered was the difficulty to purify full length p53 in significant quantity in order to perform the binding assays. Full length p53 was notorious for being prone to aggregation during purification from bacteria expression system. The experiment was limited to using a soluble fragment of p53 containing the DNA binding domain alone, which did not show robust binding to the #147 compound. (5) Researchers have completed a round of high-throughput screen to identify new p53 regulators using a cell-based assay. To date approximately 110,000 compounds have been screened and 230 initial hits have been selected and undergoing further testing in the lab. High throughput drug screens are inherently risky endeavors and whether these hits contain specific p53 regulators will be scrutinized using multiple functional assays.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program

Appendix D

Fiscal Year 2021-2022 Active Grants

Funded Fiscal Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
9BC03	Florida State University	Jennifer Steiner, PhD	$732,238.00	11/30/22	No	Yes	No
9BC04	Florida State University	George Rust, MD, PhD	$800,487.00	11/30/22	No	Yes	No
9BC07	H. Lee Moffitt Cancer Center and Research Institute	Gina M. DeNicola, PhD	$1,335,000.00	5/31/24	No	Yes	No
9BC08	H. Lee Moffitt Cancer Center and Research Institute	Nelli Bejanyan, MD	$1,335,000.00	4/30/26	Yes	No	No
9BC13	University of Miami	Kerry L. Burnstein, PhD	$801,000.00	10/31/22	No	Yes	No
9BC14	University of South Florida	Hong Yuan (Rays) Jiang, PhD	$801,000.00	10/31/22	No	Yes	Yes

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

Principal Investigator: Jennifer 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 approximately 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 placed under the skin of 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 that the patient gets cancer versus continuing to drink. In this year of the project the first aim has been completed in its entirety and is being prepared for publication and the second aim is over halfway completed with one cohort of animals remaining to be completed along with tissue analyses. Thus far the data has shown that consuming alcohol daily and then stopping once cancer starts to develop still worsens the development of cancer cachexia and loss of muscle, however this is exaggerated in males compared with females. Similarly, drinking alcohol prior to and throughout cancer also worsens the loss of muscle mass and strength in both males and females. When chemotherapy is used to slow the growth of the tumor, alcohol consumption does not interfere with its ability to reduce tumor size and does not appear to worsen cachexia beyond that induced by alcohol and cancer alone. Once measurements for Aim 2 are complete the researchers will investigate whether exercise could be used as a way to protect the muscle from the effects of alcohol and cancer cachexia. All of 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 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 #:** 9BC04 Modeling Paths to Cancer Health Equity

Principal Investigator: George Rust, MD, PhD

Organization: Florida State University

Abstract: Breast and colorectal cancers are two of the most screenable and treatable cancers, yet both still rank in the top five for cancer deaths. While death rates for each are declining in the U.S., the 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 these communities can most strategically target local interventions to achieve the greatest impact on cancer outcomes. The researchers have 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 the conceptual model. The Bayesian modeling approach was more accurate than a traditional statistical approach. The researchers have extended the relevance of the models to predict cancer survival rates, and to distinguish variables that cannot 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 would we save?" Or, "should we spend resources on more mammography testing, or should we focus on eliminating racial differences in access to cutting-edge treatments?" Validation of the models is ongoing on Florida data and moving to county-level predictions using state cancer registries through the Florida Cancer Data System. Community stakeholders have been engaged and listening sessions through videoconference and telephone focus groups conducted with African American persons in five regions of Florida. The researchers are now seeking to understand how best to present 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 application development. Application developer-designers with the Florida Resources and Environmental Analysis Center at Florida State University have built the framework for a web portal in parallel with the model-building and community engagement. The researchers are simplifying analytics to allow for interactive data visualization, to create and test the best formats for presenting decision tools for racial disparities in breast cancer across Florida. Ultimately, it is hoped the computer models, apps, and visualization tools will provide is the ability for Florida community stakeholders to say: "In my community, we could save x number of lives or prevent the suffering of late-stage cancers for xx people if we focused on this (better screening, or quicker referral to diagnostic biopsy or better access to cutting-edge cancer treatments)."

Follow on Funding: None at the time of reporting.

Collaborations: Researchers have built an effective inter-disciplinary, collaborative team including faculty at the FSU College of Medicine and the FSU Department of Statistics, with high-level academic researchers in the disciplines of medicine, public health, health services research, systems engineering, computer modeling, biostatistics, and geospatial analysis.

Researchers have incorporated PhD and MD students in the team to provide learning opportunities and to train the next generation in team science approaches to real-world challenges. These students are actively contributing to discussion, literature review, and manuscript writing.

Journals: Luo Y, Carretta H, Lee I, LeBlanc G, Sinha D, Rust G. Naïve Bayesian network-based contribution analysis of tumor biology and healthcare factors to racial disparity in breast cancer stage-at-diagnosis. Health Inf Sci Syst. 2021 Sep 24;9(1):35. doi: 10.100

Patents: None at the time of reporting.

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

Principal Investigator: Gina M. DeNicola, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: Lung cancer is the leading cause of cancer-releated death. Mutations in the NRF2/KEAP1 circuit are among the most common mutations in lung cancer, are suggested to cause chemo/radioresistance, 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 for Floridians and across the country. Aim: Target NRF2-regulated metabolism for cancer therapy research has resulted in a publication describing a combination strategy to kill tumors based on small molecule therapeutics that are "NQO1-activatable" to generate reactive oxygen species (free radicals), 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 researchers have performed a genetic screen and identified 2 additional targets, which have now been validated. The researchers have published the results of one of these targets, called Superoxide Dismutase2 (SOD2). Researchers found that inhibition of SOD2 results in a failure of cancer cells to maintain their energy when treated with the free radical generating compound, and enhanced cell death. In parallel studies, it was also found that cancer cells with NRF2/KEAP1 mutations are sensitive to starvation of the metal copper because the cells have lower levels of copper. These studies also suggest these cells are sensitive to copper overload as well. The researchers are currently performing studies to understand why and how the cells can leverage this information for therapy. Aim 2: 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. The analysis of KEAP1/NRF2 mutation status with radiation response has been completed. For determining chemotherapeutic response, patients were identified by leveraging Moffitt's enterprise wide data warehouse. Cohorts for the analysis of chemotherapy response were assembled and sequencing of NRF2 and KEAP1 on the full cohort was performed. Analysis of the association of KEAP1/NRF2 mutation status with chemotherapy response is currently ongoing. Next steps will be initiate the immunotherapy studies.

Follow on Funding: None at the 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. He is analyzing the association between KEAP1/NRF2 mutations and tumor response to radiation. Dr. Paul Hergenrother, department of Chemistry, University of Illinois at Urbana-Champaign to obtain IB-DNQ.

Journals: Ge EJ, Bush AI, Casini A, Cobine PA, Cross JR, DeNicola GM, Dou QP, Franz KJ, Gohil VM, Gupta S, Kaler SG, Lutsenko S, Mittal V, Petris MJ, Polishchuk R, Ralle M, Schilsky ML, Tonks NK, Vahdat LT, Van Aelst L, Xi D, Yuan P, Brady DC, Chang CJ. Connecting copper and cancer: from transition metal signalling to metalloplasia. Nat Rev Cancer. 2022 Feb;22(2):102-113. doi: 10.1038/s41568-021-00417-2. ePublication 2021 Nov 11. PMID: 34764459; PMCID: PMC8810673.

Jiang C, Ward NP, Prieto-Farigua N, Kang YP, Thalakola A, Teng M, DeNicola GM. A CRISPR screen identifies redox vulnerabilities for KEAP1/NRF2 mutant non-small cell lung cancer. Redox Biol. 2022 Aug;54:102358. doi: 10.1016/j.redox.2022.102358. ePublication 2022 Jun 2. Erratum in: Redox Biol. 2022 Jul 3;:102393. PMID: 35667246; PMCID: PMC9168196.

Patents: None at the time of reporting.

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

Principal Investigator: Nelli Bejanyan, MD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: The research group recently reported that patients with European Leukemia Net (ELN) 2017 adverse genetic risk acute myeloid leukemia (AML) have higher rates of leukemia recurrence (about 40%) and poor survival after stem cell transplantation (SCT) compared to those who have favorable or intermediate risk AML (Hansen/Bejanyan et al. TCT 2021; PMID:33781526). ELN 2017 genetic risk is considering chromosome abnormalities and genetic mutations within the leukemia cells to determine the AML risk category. The researchers' study findings have been subsequently validated in a larger registry study of AML patients receiving SCT (Jimenez Jimenez et al BMT 2021, PMID: 34584240). Based on this findings the research group recently revised the ongoing clinical trial protocol "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" (NCT05015426) to use this validated ELN 2017 adverse risk as study inclusion criteria instead of originally proposed measurable residual disease (MRD) positive AML. This is because MRD is not yet validated for AML SCT outcome. This is very important, since the research group's currently available trial is designed to use administration of large number of anticancer healthy donor cells (called γδ T cells) to treat ELN 2017 adverse risk AML patients who are at the highest risk of leukemia recurrence and death. While this trial is still ongoing, if this anticancer cell treatment is determined to be safe and successful to treat AML, this γδ T cell treatment can cure many patients with high risk AML. Estimated AML related deaths in the US were 11,400 in 2021. This trial has potential to benefit many Floridians in particular as AML is the most common acute leukemia in adults and the frequency of it increases with age. Approximately 40% of Floridians are 50 years and older and thus AML is more commonly seen among Floridians.

Follow on Funding: Merit Society Award, Start date; 4/21/22, Tampa Merit Society females supporting a research of female oncologists, $20,000

Collaborations: CareDx Pharma will be funding additional correlative studies, which include measurable residual disease screening of AML by next generation sequencing (NGS) and serial NGS-based more sensitive chimerism (includes also CD34 chimerism) monitoring throughout the study's one year follow time.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

5. **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 2022, 20,680 men will be diagnosed with prostate cancer (PC) and 2,720 will die of this malignancy, per American Cancer Society estimates. Advanced PC tumors 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 therapies specifically tailored 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. This project exploited these data to identify and prioritize drugs and drug combinations to treat PC more effectively. Significant progress was made in three major areas: (1) the computational framework for evaluating new therapeutics was launched and expanded based on PC tumor data; (2) new compounds (identified by computationally screening thousands 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); and (3) complimentary advanced machine learning approaches to predict drug sensitivities were developed. Available data from 520 PC patient tumors and 71 non-cancer prostate samples was processed. PC-specific gene "signatures" were identified for computational screening against the constellation of drugs and combinations. This method produced "modules" that identify classes of genes that are over-represented in large numbers of PC patients and are associated with disease state. An integrated database was developed to predict and prioritize effective PC therapeutics. A multi-pronged approach combined live cell imaging (measuring cell growth and cancer-like changes) and endpoint assays (determining drug-induced cellular toxicity) to define PC sensitivities to individual drugs and combinations. Experimental throughput was accelerated by employing liquid-handling robotics (purchased previously with university funds not derived from this Bankhead-Coley award). Fifty-four drugs were tested against a panel of cell lines (PC, advanced PC, and non-cancer prostate tissue). "RNAseq" data characterized "deep" genetic differences between cell lines. Many of these compounds are 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 individual drugs. Two main compound groups were identified: (1) compounds that reduced viability across all evaluated PC cell lines independent of their biological and genetic differences; and (2) compounds that specifically inhibited certain PC cell lines but not others. Experimental results from tested compounds were used in a computational screening pipeline to sharpen the resolution of its predictions. In parallel, machine-learning approaches were developed to predict PC cell sensitivities to drugs

— strategies now being extrapolated to human precision medicine applications. Drugs predicted by both the module-based connectivity and machine learning approaches are being tested in PC cell lines and more complex models such as human tumors transplanted to mice. Extensive data on PC has already been curated and will be made publicly available as a permanent resource for the PC research community and to maximize the project's impact. This technology may be further developed into a tool that prioritizes drug treatments for PC patients.

Follow on Funding: None at the time of reporting.

Collaborations: Kerry Burnstein, PhD, University of Miami (PI), Stephan Schürer, PhD, University of Miami, Vasileos Stathias, PhD, University of Miami, Rimpi Khurana, PhD, University of Miami, Beronica Ocasio, University of Miami, Maria Julia Martinez, PhD, University of Miami, Nahuel Peinetti, PhD, University of Miami, Benjamin Sherman, University of Miami.

Journals: Khurana R, Schürer S. The Clinical Kinase Index (CKI): A user friendly application to prioritize kinases as prospective cancer drug targets. Software Impacts, 2022; 12:100257. doi.org/10.1016/j.simpa.2022.100257.
Issa NT, Stathias V, Schürer S, Dakshan

Patents: None at the time of reporting.

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

Principal Investigator: HongYuan (Rays) Jiang, PhD

Organization: University of South Florida

Abstract: The research team made the exciting discovery that cancer cells use an imbalanced heme biosynthetic pathway and compensate for this "insufficiency" with increased heme trafficking. This process is called "heme overdrive". Heme is an iron-containing molecule with various cellular functions. Cancer cells have high levels of heme precursor molecules (called porphyrins) and increased heme transport. Heme overdrive may provide an ideal anti-cancer target, as it is: 1) cancer universal (present in all cancers), 2) cancer essential (cancer cells require it), and 3) cancer specific (absent in normal cells). The interdisciplinary team determined that: 1) metastatic cancer cells exhibit heme overdrive, based on gene editing loss-of-function studies, 2) the cancer tumor microenvironment exhibits heme overdrive, based on the researchers' experimental validation of lung cancer-associated fibroblasts isolated from treatment-naïve patients, 3) end-stage solid tumors have elevated heme overdrive, and 4) cancer progenitor cells have heme overdrive, while normal cells from the same patient have not, as examined using single cell analyses. These and other results indicate the exquisite cancer specificity of heme overdrive. To translate these research findings, the researchers designed a bait-and-kill strategy that utilizes heme overdrive to sensitize cancer cells to cytotoxic drugs. Cancer cells can be sensitized, by using heme overdrive to induce porphyrin levels ("baiting"), to be "killed" by drugs that attack the glutathione antioxidant system, a cell defense mechanism that protects cells from the oxidative stress induced by porphyrin accumulation, or by drugs that further increase oxidative stress. The bait-and-kill strategy has no detectable toxicity in primary non-cancerous lung cells, or any other healthy cells tested in the lab. It is expected that the outcome of these studies will provide proof-of-concept results showing that aberrant heme metabolism is uniquely present in cancer cells and provides a novel therapeutic target. The potential long-term impact of these innovative studies is the development of an effective anti-

cancer therapy that is significantly less toxic than previous therapies used to target cancer metabolism.

Follow on Funding: None at the time of reporting.

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

Journals: None at the time of reporting.

Patents: None at the time of reporting.

William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program
Appendix E
Fiscal Year 2021-2022 Closed Grants
Funded Fiscal Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
9BC01	Florida Atlantic University	Esther A. Guzman, PhD	$801,000.00	5/31/22	No	No	No
9BC09	H. Lee Moffitt Cancer Center and Research Institute	Ernst Schonbrunn, PhD	$800,454.00	3/31/22	No	Yes	Yes
9BC12	University of Miami	Anthony Capobianco, PhD	$801,000.00	4/30/22	No	No	No

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

Principal Investigator: Esther A. Guzman, PhD

Organization: Florida Atlantic University

Abstract: Breast cancer is the second leading cause of cancer death in women. Triple negative breast cancers (TNBC) represent about 12% of breast cancers and are the most aggressive and difficult to treat. The uniqueness, chemical diversity and structural complexity of marine natural products represent an unexploited supply of potential new drugs, lead compounds for medicinal chemistry optimization or biological probes to allow for better understanding of diseases. The unique library of pure and highly enriched fractions derived from marine organisms that are used in this project contains fractions/compounds derived from deep-water marine invertebrates that are not readily available outside of HBOI. The research team hypothesized that compounds with the novel activity of inducing cytotoxicity in TNBC cell spheroids while not being toxic to cells grown in traditional 2D cultures can be found among secondary metabolites from marine organisms and that these compounds will have the potential to be novel therapeutics for the treatment of TNBC or as probes to further the understanding of this kind of cancer. Cancer cells grown in spheroid conditions (3D cultures) allow the cells to interact with each other and the extracellular matrix providing a better representation of the in vivo environment than 2 dimensional cultures. Successful completion of the proposed research will lead to the identification of compounds which selectively kill TNBC cells grown in 3D culture and insight into proteomic and genomic cellular effects elicited by the top active compounds.

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 #:** 9BC09 Development of Novel TAF1 Inhibitors

Principal Investigator: Ernst Schonbrunn, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: TATA-Box Binding Protein Associated Factor 1 (TAF1) is a potential target to develop small molecule therapeutics for diseases arising from dysregulated transcription such as cancer. Researchers discovered that the ataxia telangiectasia and Rad3-related (ATR) kinase inhibitor AZD6738 (ceralasertib) is a bona fide inhibitor of the tandem bromodomain of TAF1. We synthesized a series of AZD6738 analogues and studied the effect of these dual TAF1-kinase inhibitors on p53 signaling and DNA damage response in blood and solid cancer cell line models. Along with the previously reported TAF1 inhibitors BAY299 and GNE371, researchers characterized the structural basis of TAF1 tandem bromodomain inhibition by small molecules using X-ray crystallography and small-angle X-ray scattering.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Rezaul Md Karim, Leixiang Yang, Lihong Chen, Melissa J. Bikowitz, Junhao Lu, Dylan Grassie, Zachary P. Shultz, Justin M. Lopchuk, Jiandong Chen, Ernst Schönbrunn. Discovery of dual TAF1-ATR inhibitors and ligand-induced structural changes of the TAF1 tandem bromodomain.

Patents: Patent application PCT/US21/23419 Title: TAF1 inhibitors Inventors: Ernst Schonbrunn, Justin Lopchuk, Rezaul Karim Institution: Moffitt Cancer Center provisonal patent application MCC 10110-327PV1Title: Compounds for targeted degradation of TAF1 Inventor

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

Principal Investigator: Anthony Capobianco, PhD

Organization: University of Miami

Abstract: The research team discovered three most potent and selective compounds (BC-57, BC-45 and BC-14) that target the BCL9/β-catenin interface and disrupt Wnt/β-catenin mediated transcriptional activity. Of the three scaffolds, BC-57, were prioritized for further development. Researchers optimized various parameters of the BC-57 scaffold in parallel with developing structure-activity relationships (SAR). The team has carried out SAR studies on approximately 300 derivatives of the lead scaffold. Optimization parameters include potency and pharmacokinetic properties of the BC-57 derivatives. Optimization of the scaffold has allowed changes to multiple positions at various sites on the compound.

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.

William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program
Appendix F
Fiscal Year 2021-2022 Closed Grants
Funded Fiscal Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
8BC01	Mayo Clinic Jacksonville	John A. Copeland, PhD	$815,283.00	10/31/20201	No	Yes	No

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

Principal Investigator: John A. Copeland, PhD

Organization: Mayo Clinic Jacksonville

Abstract: The research team developed and patented composition of matter of a novel small molecule inhibitor of stearoyl CoA desaturase 1(SCD1) called SSI-4. SSI-4 blocks monounsaturated fatty acid (MUFA) synthesis. These fatty acids are important in causing tumor growth and drug resistance. The team discovered that these MUFAs may also interact with immune cells to further promote tumor growth. Using these fairly new FDA drugs called immune checkpoint inhibitors (ICIs; anti-PD1 and anti-PD-L1 antibodies) that target immune cells, the team showed that an ICI combined with SSI-4 proved very effective in blocking tumor growth in triple negative and HER2 positive breast cancers. This can lead to new and more effective treatment for these patients who may otherwise die.

Follow on Funding: None at the time of reporting.

Collaborations: Mayo Clinic, Cancer Biology and Immunology Departments and Hematology/Oncology Division, Internal Medicine Department, Jacksonville FL. Post-doctoral Fellows: Drs. Sneha Vivekanandhan, Justyna Trynda (Gleba/married name), Barath Shreeder Hem/Onc. Chief.

Journals: Pope ED 3rd, Kimbrough EO, Vemireddy LP, Surapaneni PK, Copland JA 3rd, Mody K. Aberrant lipid metabolism as a therapeutic target in liver cancer. Expert Opin TherTargets.2019Jun;23(6):473-483.doi:10.1080/14728222.2019.1615883. ePublication 2019May10.PMID:31076001;

Patents: None at the time of reporting.

William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program
Appendix G
Fiscal Year 2021-2022 Closed Grants
Funded Fiscal Year 2016-2017

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
7BC03	University of Miami	Emmanuel Thomas MD, PhD, FAASLD	$1,866,436.00	2/28/22	Yes	No	No
7BC04	H. Lee Moffitt Cancer Center and Research Institute	Gwede K. Clement, PhD, MPH, RN, FAAN	$828,125.00	2/28/22	No	Yes	No

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, PhD, FAASLD

Organization: University of Miami

Abstract: First, the researchers would like to thank the Bankhead-Coley program for their support. The greatest achievement for the last year was having the state of FL be recognized for its efforts to eliminate HCV(https://www.hhs.gov/hepatitis/get-involved/hepatitis-elimination/index.html). Furthermore, the research team has created the HCV free Florida website (www.HCVFreeFL.com) and an associated Twitter account to support HCV elimination efforts in FL. Through this funding, the team was 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 . The team have 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 research 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 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 initial efforts to develop a liver cancer risk calculator that utilizes race/ethnicity, the researchers have 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, researchers believe these efforts will lay the foundation for future work. Using multivariable statistical modeling, the researchers are 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, follow on grants to the National Institutes of Health (NIH), Department of Defense and a Bankhead Infrastructure grant have been submitted that will take this 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 5-year, renewable grant from the NIH for $1.9 million. This NIH funded study is focused on understanding inflammatory mechanism that lead to chronic viral infections in the liver through basic science laboratory studies. The grant is a nice complement to this clinical study, supported by the Florida Department of Health, and the funding from this grant has increased since the researchers subsequently received a minority supplement to support a graduate student. In addition, the PI has been awarded and renewed a $300,000 grant from Gilead Sciences to

screen for HCV and HIV in the University of Miami Emergency department. Furthermore, the researchers recently established the Florida HCV-HCC/Liver Cancer Consortium with Moffitt Cancer Center, University of Florida and Jacksonville Mayo Clinic.

Follow on Funding: Gilead Sciences, 7/1/22-6/30/23, $167,188

Collaborations: This project is being performed at the University of Miami Miller School of Medicine. Furthermore, research staff recently formed the Florida HCV-HCC/Liver Cancer Consortium with Tampa Moffitt Cancer Center (Dr. Anna Giuliano-Center for Infectious Cancers), University of Florida Gainesville (Drs. David Nelson-Hepatology and Betsy Shenkman-Medicine) and Jacksonville Mayo Clinic (Dr. Tushar Patel-Transplant Hepatology) through an initial meeting that was held at Moffitt Cancer Center in Tampa in October 2017. The next meeting was held in Miami on May 7th 2018 and Dr. Emmanuel Thomas co-lead that meeting in Miami. The most recent meeting was at the University of Florida in Gainesville on April 19th, 2019 and the next meeting took place in Orlando on August 16th, 2019. It is anticipated that additional grants will be submitted with work from this multi-institutional group that is focused on liver cancer/HCC. This project is currently providing training to four University of Miami Graduate students: Dennis McDuffie (fourth year graduate student-PhD program), Jasmine Edwards (fifth year graduate student-PhD program) and Alejandro Badilla (4th year graduate student-PhD program) and three University of Miami undergraduate students (David Barr, Robert DiCaprio and Justin Yu). In addition, the research team has developed and grown a new training program for interested medical students at the University of Miami specifically focused on oncology from a multidisciplinary perspective. It includes educational materials and speakers from radiation oncology, surgical oncology, medical oncology, radiology, interventional radiology, pathology, gynecologic oncology and immuno-oncology. This will greatly expand the number of students that will benefit from this state funding that is supporting the PI since there are currently over 50 individuals in the oncology training pathway for medical students.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

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

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: One of the largest disparities in cancer mortality in the United States occurs with colorectal cancer (CRC). The overall purpose of Community CARES (Colorectal Cancer Awareness, Research, Education and Screening) or the C-CARES Project, was to test an innovative multi-level intervention delivered in Federally Qualified Health Centers (FQHCs) in Southwest Florida, to promote sustained CRC screening (CRCS) with fecal immunochemical test (FIT). The effectiveness of FIT screening is predicated on consistent annual repeat screening over time, particularly given that CRC is highly detectable, preventable, and beatable when treated at early stages. Many Floridians who receive their healthcare in community-based primary care settings stand to benefit from these efforts.

The C-CARES project has progressed well and has met specified scientific goals. There were two study arms: C-Cares (education + FIT) vs. C-Cares Plus (education + FIT + personalized

components) among 328 individuals, 50-75 years of age, who weren't up-to-date with CRC screening. The research team assessed initial CRC screening uptake and annual adherence. Core accomplishments included: 1) continuation of Community Advisory Board (CAB) meetings; 2) concluded all follow-up activities, which entailed navigation of patients needing colonoscopies (after abnormal FIT kit results), coaching of participants who did not return their FIT kits (C-CARES plus arm), and follow-up to assess annual repeat FIT screening; 3) conducted initial analyses of outcomes; and 4) began dissemination of results.

Initial results showed that 69% of the 328 participants had returned their first FIT kit by 6 months post intervention. A total of 65 patients over the study period had an abnormal FIT result, and the majority have successfully completed follow up colonoscopies. No cancers were diagnosed among these. Initial results have been published (6 months post intervention) and another publication is in preparation to report screening rates for annual repeat screening.
Christy SM, Sutton SK, Abdulla R, Boxtha C, Gonzalez P, Cousin L, Ewing A, Montoya S, Lopez D, Beehler T, Sanchez J, Carvajal R, Meade CD, Gwede CK. Main Outcomes Publication: C-CARES main outcomes is in press: A multilevel, low literacy dual language intervention to promote colorectal cancer screening in community clinics in Florida: A randomized controlled trial. Prev Med. 2022 Mar 16:107021.
https://pubmed.ncbi.nlm.nih.gov/35305995/

The produced CRC English/Spanish educational materials have been adopted by clinic partners and are benefiting high numbers of Florida patients. The clinics have adopted best practices for distribution of free FIT tests and providing linkages to colonoscopy after an abnormal FIT test. The achieved initial FIT screening rate of 69% matches the state's general population CRC screening average (approximately 67%) and far exceeds the rate of approximately 39-40% (HRSA data: the U.S. Department of Health & Human Services Health Resources & Services Administration) seen in Florida's Federally Qualified Health Centers (FQHC). These findings suggest high potential for scale-up and offer lessons for disseminating effective strategies to other FQHC's or to address other recommended cancer screenings.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Christy SM, Sutton SK, Abdulla R, Boxtha C, Gonzalez P, Cousin L, Ewing A, Montoya S, Lopez D, Beehler T, Sanchez J, Carvajal R, Meade CD, Gwede CK. Main Outcomes Publication: C-CARES main outcomes is in press: A multilevel, low literacy dual language intervention to promote colorectal cancer screening in community clinics in Florida: A randomized controlled trial. Prev Med. 2022 Mar 16:107021.

Patents: None at the time of reporting.

William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program

Appendix H
Fiscal Year 2021-2022 Closed Grants
Funded Fiscal Year 2015-2016

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
6BC09	University of Florida	Walter O'Dell, PhD	$1,310,643.00	2/28/22	Yes	Yes	No

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

Principal Investigator: Walter O'Dell, PhD

Organization: University of Florida

Abstract: This project was focused on better documenting and understanding the biological mechanisms and factors that influence radiation damage to the lung in breast cancer patients. Athought 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 researchers are intersted 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 quanitfy both tissue and vasculary damage from CT scans of the chest, and will correlate these changes with clinical findings of lung distress and patient survival, with pulmonary function (breathing) tests, blood markers of tissue toxicity, and quality of life surveys. The research team enrolled 41 breast cancer patients with 38 providing complete or near-complete data. For each subject, baseline was acquired with 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 researchers have 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. Researchers validated this method using three-dimensional print-out of a real lung arterial tree that we extracted from the chest CT scan of human volunteer. Student researchers manually measured the radius of 69 branches in this 3D printed tree. The researchers then endeavored to calibrate the vessel counts for different pixel sizes and reconstruction filters. The last few chest CT scans are currently being analized. An initial analysis showed that smoking history greatly impacts the number of blood vessels in the lung and their changes after radiation. Meanwhile, analysis of the serial blood samples for all patients has been completed, focusing on 13 key markers of tissue injury. Statistical modeling showed that the 13 markers can be reduced to 4 groups that independently relate to patient and treatment factors. Presumably, each group represents a unique response pathway where multiple markers are associated. A preliminary analsyis 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 their body mass index (BMI). Elevated BMI results

in higher concentration of this markers after chemotherapy, and greater change after radiation treatment. The 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: Florida Breast Cancer Foundation, 8/1/2021, $99,450

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;10(1):10559. do

Patents: None at the time of reporting.

James and Esther King Biomedical Research
Appendix I
Fiscal Year 2021-2022 Newly Awarded Active Grants
Funded Fiscal Year 2021-2022

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
22K01	Mayo Clinic Jacksonville	Owen Ross, PhD	$1,453,280.00	3/31/25	No	No	No
22K02	H. Lee Moffitt Cancer Center and Research Institute	Jennifer Vidrine, PhD	$1,451,330.00	9/30/26	No	No	No
22K03	University of Florida	Bently Doonan, MD, MS	$1,458,000.00	9/30/26	No	No	No
22K04	University of Florida	Brian Law, PhD	$98,940.00	9/30/22	Yes	Yes	Yes
22K05	University of Florida	Daiqing Liao, PhD	$583,200.00	3/31/25	No	No	No
22K06	University of Miami	Nagaraj Nagathihalli, PhD	$583,200.00	3/31/25	No	Yes	No
22K07	University of Miami	Roberto Vazquez-Padron, PhD	$583,200.00	3/31/25	No	No	No
22K08	University of South Florida	Ji Li, PhD	$583,200.00	3/31/25	Yes	Yes	No
22K09	University of South Florida	Mohapatra Subhra, PhD	$100,000.00	9/30/22	No	No	No

1. **Grant #:** 22K01 Creating a Florida Cerebrovascular Disease Biorepository and Genomics Center

Principal Investigator: Owen Ross, PhD

Organization: Mayo Clinic Jacksonville

Abstract: The Global Burden of Disease Study ranked cerebrovascular disease as the second leading cause of death. Stroke is associated with significant morbidity and mortality. Standardizing phenotypic data collection and identifying genetic variants that determine risk for cerebrovascular diseases will offer new insight into the development of these conditions and may provide personalized treatment for these diseases. The research team has established the Mayo Clinic Florida Cerebrovascular Diseases Registry (CDR) to build a biorepository of specimens from patients who enter the clinic.

The team has previously collected a series of 1000 patients (53% female; mean age, 60.4 years), including over 200 from underrepresented minority communities. These patients undergo extensive clinical phenotyping. The registry classifies patients by one or more of 23 cerebrovascular conditions as well as concurrent stroke-free healthy control subjects. Eligible conditions include cerebral infarction, cerebral hemorrhage, ruptured and unruptured cerebral aneurysms, and cavernous malformations. Each eligible cerebrovascular condition has strict requirements for evidence of the disease based on imaging, laboratory results, or diagnostic guidelines. Participants undergo a structured intake assessment of demographic variables, stroke risk factors, stroke symptoms, neurological impairment, mental status, and functional status pre- and post-symptom onset. A detailed family history is obtained and affected family members are encouraged to participate.

The goal of the proposed studies will be to expand the registry, not only based on Mayo Clinic Florida samples, but also to other academic medical centers in Florida. The research team will propose DNA sequencing in collected samples to build a genomic sequencing-bioinformatics pipeline for studies of cerebrovascular disease in Florida. In addition, over 10% of the samples collected thus far are from minorities including African American and Hispanic populations. The team has already started genetic analysis in these populations and propose to prioritize collection and analysis in these cohorts.

Mayo Clinic Florida was the first center in Florida to receive the Advanced Certification as a Comprehensive Stroke Center by The Joint Commission. This distinction identifies centers that are focused on providing advanced and complex stroke care. Collaborators from the University of Miami and the University of Florida will provide additional support for this research infrastructure by aiding in the recruitment of diverse populations with young onset and familial cerebrovascular disease. Overall, Mayo team plans on initiating a multi-center biospecimen repository for cerebrovascular disease that will expedite the identification of novel variants implicated in cerebrovascular disease and lead to the development of prognostic tools, advanced therapies and treatment, and improved techniques for early detection methods. To date, the study has received institutional review board approval from the Mayo Clinic board to move forward with subject recruitment and meetings held with both external collaborators to initiate approval and collection. Also, the research team has begun to identify patients with Cerebral Autosomal Dominant Arteriopathy with Sub-cortical Infarcts and Leukoencephalopathy (CADASIL) as a rare cerebrovascular disease with cognitive impairment, the researchers believe starting with this disease will facilitate the establishment of the overall state registry.

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 #:** 22K02 Enhancing Long-Term Smoking Abstinence Among Cervical Cancer Survivors

Principal Investigator: Jennifer Vidrine, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: This project is evaluating a novel, personally-tailored text-based treatment as an adjuvant to the empirically-based Motivation and Problem Solving (MAPS) counseling approach among cervical cancer survivors and individuals with high grade dysplasia. The overall purpose of the study is to determine if this smoking cessation strategy is efficacious in facilitating long-term smoking abstinence.

To date, the research team submitted the study protocol and relevant materials and obtained full-board approval from the Institutional Review Board (IRB), Advarra in April 2022. The primary progress has been focused on the development and refinement of the text-based treatment adjuvant content, which is intended to enhance the long-term efficacy of the MAPS phone counseling intervention, for the MAPS+ treatment group. The content was developed and reviewed by the research team, including the Principal Investigator (PI), Co-Investigators, and research project staff.

In addition to the development of the text-based intervention content, the research team finalized vital study forms and initiated the design and development of the electronic database using Moffitt's Research Electronic Data Capture (REDCap). It is anticipated that the research project staff finalized the database and conducted relevant quality assurance testing by the end of August 2022.

Finally, the research project staff are collaborating with Moffitt's marketing firm to develop a social media-based participant recruitment campaign, which will be launched in September 2022.

Because the research project staff have not yet begun enrolling participants in the study, the study has not yet had a direct impact to Floridians. However, the potential to reduce tobacco-related cervical cancer morbidity and mortality in Florida as the study progresses is exceedingly high.

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 #:** 22K03 Novel RNA-Nanoparticle Vaccine for Treatment of Early Melanoma Recurrence Following Adjuvant Anti-PD-1 Antibody Therapy

Principal Investigator: Bently Doonan, MD, MS

Organization: University of Florida

Abstract: Melanoma is an increasing public health concern in the state of Florida with 7900 new diagnoses annually. The advent of immune checkpoint inhibitors (ICI) has revolutionized the treatment of advanced cutaneous and mucosal melanoma. Based on their profound impact on advanced disease, immune checkpoint inhibitors have also become the standard of care in the adjuvant setting in resected stage III/IV melanoma following surgery. These agents have shown a prolonged recurrence-free survival in these high-risk melanoma patients, however, up to 30% of patients will have disease recurrence within 1 year of treatment. Previous studies have also shown that patients who develop disease recurrence following adjuvant ICI treatment, or while on treatment, have a more aggressive course of disease that does not respond to PD-1 inhibitors and has a poor response to CTLA4 inhibition. Furthermore, patients with mucosal melanoma who had recurrence did not benefit from subsequent ICI therapy at all, highlighting this subtype of melanoma as an area of high unmet clinical need. One reason for the failure of ICI in the post adjuvant setting is the immune suppressive nature of the melanoma tumor microenvironment (TME) and lack of professional antigen presenting cell (APC) activation. These APCs, namely dendritic cells (DC) often remain in an inert state unable to present tumor antigens for immune detection due to lack of innate immune cell triggering and inhibition from myeloid derived suppressor cells (MDSCs). This innate arm of the immune system works to prime and drive the natural immune response to both virally infected cells and against malignancy which then activates the adaptive immune T cell response against the tumor. The team has developed a novel (FDA approved) RNA-nanoparticle (RNA-NP) vaccine that simultaneously penetrates and reprograms the TME while inducing a tumor specific adaptive T cell response. This vaccine utilized novel engineering design that layers tumor derived mRNA into a lipid-nanoparticle (NP) "onion-like" package. These NPs enable maximal isolated and stable packaging of mRNA into a fixed volume for easy distribution, cellpenetration, and uncoating which quickly boosts innate and adaptive immune responses. These RNA-NPs localize to the TME and activate multiple innate pathways thereby activating DC and suppressing the function of MDSCs. In this study, researchers propose the use of patient

derived RNA-NP vaccine in patients with early recurrence of melanoma who previously received or are currently receiving PD-1 therapy. The team proposes that through re-priming of the antitumor immune response and alteration of the TME that researchers can improve the efficacy of PD-1 therapy and prevent the need for subsequent lines of therapy which have little current clinical value. If effective, this treatment will revolutionize the management of this aggressive subset or melanoma patients and improve overall survival. This study will also gather important information into the mechanisms of early ICI resistance, identify novel biomarkers of innate cell resistance and response to treatment, and provides a cutting edge, personalized immunology approach to melanoma treatment.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

4. **Grant #:** 22K04 Toward IND-Enabling Studies for Novel Cancer Therapeutics that Inhibit the Disulfide Isomerases ERp44, PDIA1, and AGR2

Principal Investigator: Brian Law, PhD

Organization: University of Florida

Abstract: The goal of this bridge-grant project is to obtain information key to increase intellectual property on a new class of drugs discovered by this research team, that have activity in animal models of patient-derived breast cancer.

The research team's work on the anti-cancer compounds Disulfide bond Disrupting Agents (DDAs). Is novel in several respects. DDAs are chemically different than all existing classes of anti-cancer agents. DDAs are the first identified inhibitors of the Protein Disulfide Isomerase (PDI) enzymes ERp44 and AGR2. While AGR2 is well known to promote tumor growth, ERp44 has not previously been investigated as a target of anti-cancer agents. DDAs rapidly induce the death of cancer cells in primary breast tumors and metastases without harming normal tissues. DDA inhibition of their PDI targets causes activation and upregulation of Death Receptor 5 (DR5). While DR5 is known to selectively kill cancer cells, extracellular ligands have not been FDA approved due to several resistance mechanisms. Finally, DDAs bypass several of the resistance mechanisms observed with previous activators of DR5.

To date, progress on the project has involved synthesis of DDA compounds for tumor studies and generating patient-derived xenograft (PDX) tumor models in mice with which to perform studies of DDA anti-cancer efficacy. The research team has obtained a quote for performing pre-clinical pharmacological studies (half-life and metabolism) that will be completed as soon as the DDA compound is ready. Additionally, the team has recently published two journal articles on the project that detail the biochemical mechanisms of DDA anti-cancer action and the chemical mechanisms that dictate DDA target protein selectivity/specificity.

Follow on Funding: Florida Breast Cancer Foundation, Brian law, PhD, 7/1/22-6/30/23, Breast Cancer Research funding, Funded: $100,000

Collaborations: Students and Postdoctoral Fellows at UF Receiving Training under Project: Dr. Amanda Ghilardi earned her Ph.D. in Dr. Ronald Castellano's laboratory and published papers on the project. B. Law was a member of Dr. Ghilardi's Ph.D. advisory committee.

Journals: Ghilardi, A., Yaaghubi, E., Ferreira, R., Law, M., Yang, Y., Davis, B., Schilson, C., Ghiviriga, I., Roitberg, A., Law, B. K.*, and Castellano, R.* 2022, Anticancer Agents Derived from Cyclic Thiosulfinates: Structure-Reactivity and Structure-Activity Relationships, ChemMedChem, May 1. DOI: 10.1002/cmdc.202200165. Online ahead of print. *Co-Corresponding Authors; Categorized as a “Very Important Paper”

Law, M., Yaaghubi, E., Ghilardi, A., Davis, B., Ferreira, R., Koh, J., Chen, S., DePeter, S., Schilson, C., Chi-Wu, C., Heldermon, C., Nørgaard, P., Castellano, R., and Law, B., 2022, Inhibitors of ERp44, PDIA1, and AGR2 induce disulfide-mediated oligomerization of Death Receptors 4 and 5 and cancer cell death, Cancer Lett., 534, 215604

Patents: Novel small molecule anticancer agents, Patent No.: US 10,813,904, Date of Patent: Oct. 27, 2020 inhibition of the PDI family members AGR2, PDIA1, and ERP44 for therapeutic treatment and use in predictive diagnotistics/monitoring for treatment.

5. **Grant #:** 22K05 Development of First-in-Class Hdac3-selective Degraders For Breast Cancer Therapy

Principal Investigator: Daiqing Liao, PhD

Organization: University of Florida

Abstract: Breast cancer (BC) is the leading cause of cancer burden for women, diagnosed in over 1 million worldwide each year. BC affects one in 20 women globally and as many as one in eight in Western countries. Although more and more patients with BC have survived of the disease, over 450,000 patients die of this disease annually. About one third of invasive BCs progress to recurrent or metastatic disease, and approximately 90% of BC deaths are due to metastatic cancer. There are several major breast cancer subtypes: estrogen receptor-positive (ER+), human epidermal growth factor receptor 2 (HER2)-enriched and triple-negative (TNBC). All BC subtypes can progress to distant metastases. Metastatic BC is currently incurable. The short median survival of three years for patients with metastatic BC has not significantly changed in over 20 years. Therefore, more effective treatments are urgently needed to combat BC.

A class of cellular enzymes known as histone deacetylases (HDACs) are associated with promoting BC progression and treatment resistance in BC. HDAC inhibitors (HDACi) such as entinostat and tucidinostat have shown clinical anticancer efficacy in combination with the aromatase inhibitor exemestane. This indicates that targeting HDACs are promising new therapy for treating BC. Notably, HDAC3, a specific HDAC isozyme, also has function independent of its enzymatic activity, which appears to be important for its oncogenic property. Conventional HDACi cannot block this function of HDAC3, suggesting that existing HDACi could not adequately ablate HDAC3’s oncogenic function. Therefore, novel strategies are needed to effectively inhibit HDAC3 in cancer cells. Proteolysis targeting chimeras (PROTACs) targeting various oncogenes have shown promising anticancer effects. Importantly, improved target selectivity can be achieved by converting a conventional non-selective inhibitor to a PROTAC. The research team has designed and synthesized novel PROTACs that degrade HDAC3 with a high potency and selectivity. These novel compounds potently impaired BC cell viability at a

very low drug concentration. In this application, the team proposes to optimize and validate the HDAC3 PROTACs for potency and selectivity in degrading HDAC3. The team will also determine (1) the mechanism of action of these novel HDAC3 PROTACs, (2) their in vivo drug properties, (3) their safety profiles, and (4) their anticancer and anti-metastatic efficacy in preclinical animal studies. The outcome of this project will provide critical proof-of-concept evidence for potentially translating the first-in-class HDAC3 PROTACs into the clinic for treating patients with advanced BC.

In this reporting period, significant progress toward the specific aims of this grant has been made. A manuscript has been submitted to a peer-reviewed journal. Additionally, future scientists at graduate and postdoctoral levels have been mentored to conduct cutting-edge research in this project. Ultimately this project might lead to new therapy for treating advanced BC.

Follow on Funding: None at the time of reporting.

Collaborations: Research collaboration, University of Florida College of Medicine and College of Pharmacy Nikee Awasthee: PhD, postdoctoral associate (Daiqing Liao, Mentor), University of Florida Chengcheng Meng: PhD graduate student (Daiqing Liao, mentor), University of Florida, Seth Hale: PhD graduate student (Daiqing Liao, mentor) Unversity of Florida, Yufeng Xaio, PhD, postdoctoral associate (Guangrong Zheng, Mentor), University of Florida

Journals: None at the time of reporting.

Patents: None at the time of reporting.

6. **Grant #:** 22K06 Targeting CREB to Improve Response to Immunotherapy in Pancreatic Cancer

Principal Investigator: Nagaraj Nagathihalli, PhD

Organization: University of Miami

Abstract: The contribution of cellular signaling molecules in smoking-induced pancreatic neoplasia is a nexus that is poorly understood. CREB (a cellular transcription factor) can be considered an oncogene in smokers due to its role in upregulating the secretion of factors that imbue an immunosuppressive microenvironment. The researchers defined the functional significance of CREB's contribution to pancreatic tumorigenesis, unraveled the complexity of heterogenous smoking-induced pancreatic cellular dysplasia that propels tumorigenesis, and exploited these findings to develop improved strategies to develop novel treatment. As CREB signaling is altered in pancreatic cancer, where tobacco smoking is a risk factor, these results will impact advancing progress toward cures and improving the survival of Floridians. The researchers have confirmed that pancreatic cancer immunosuppression occurs via a CREB-driven process that reprograms myeloid cells and increases cancer progression by altering the secretion of cytokines and chemokines. This evidence supports their hypothesis that the CREB is crucial for pancreatic cancer progression in smokers and amenable to therapeutic intervention. An in vivo model system has been developed to study smoking-induced CREB and confirmed validating CREB deleted mouse model in pancreatic cancer. These findings further validated that CREB deletion converts the tumor microenvironment from immunosuppressive to immunostimulatory and derails crosstalk between myeloid cells and tumor cells, restoring anti-tumor immunity.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Mehra, S., S. Singh, N.S. Nagathihalli. Emerging Role of CREB in Epithelial to Mesenchymal Plasticity of Pancreatic Cancer. Front Oncol, 12, 925687, (June 21, 2022). PMCID: PMC9253527

Patents: None at the time of reporting.

7. **Grant #:** 22K07 The Impact of Smoking in the Venous Cellular Ecosystem and its Consequences for Arteriovenous Fistula Maturation in CKD Patients

Principal Investigator: Roberto Vazquez-Padron, PhD

Organization: University of Miami

Abstract: This project started in April 2022. The focus of Aim 1 is to demonstrate that smoking-related changes in venous cell ecology increase the risk of arteriovenous fistula (AVF) failure. Accordingly, the researchers have small conditional RNA (scRNA) sequenced six basilic veins from human donors obtained upon a research agreement with the Life Alliance Organ Recovery Agency, generating a combined atlas of approximately 16,000 cells. Integrated clustering analysis revealed 8 clusters containing canonical vascular cell populations such as vascular smooth muscle cells (VSMCs), endothelial cells (ECs), and fibroblasts. Surprisingly, these veins had a significant number of resident immune cells, including pro-inflammatory macrophages, CD163- and HMOX1-expressing Mhem-like macrophages, lymphocytes, and small numbers of neutrophils and mast cells. The researchers are extending their biobank to incorporate ten veins from current smokers, ten from former smokers (smoking cessation >1 year), and 100 never-smokers. As planned, the research team expects to finish this aim by the end of this fiscal year.

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 #:** 22K08 Sirtuin 1 and Cardiovascular Impairment by Cigarette Smoking

Principal Investigator: Ji Li, PhD

Organization: University of South Florida

Abstract: Cigarette smoking is a major preventable cause of morbidity and mortality worldwide. It is estimated that >5 million people die from tobacco smoke-related illnesses each year. Smoking is a major independent risk factor for systemic injury, including atherosclerotic vascular disease, hypertension, and stroke. While the association between chronic smoking and cardiovascular disease is recognized, the underlying mechanisms are incompletely understood. There is emerging evidence that a longevity protein sirtuin 1 (SIRT1) can ameliorate systemic injury caused by cigarette smoking. Moreover, the research team revealed that SIRT1 agonists

play a critical role in cardioprotection against age-related cardiovascular injury through modulating metabolic homeostasis and inflammatory response. Thus, it is hypothesized that pharmacological SIRT1 agonists can ameliorate smoking-induced cardiovascular insults of hypertension patients via maintaining the metabolic and redox homeostasis. Age-matched, air-exposed mice will serve as nonsmoking controls. The pharmacological SIRT1 agonism will characterize the critical role of SIRT1 in ameliorating systemic injury caused by cigarette smoking exposure. In this manner, the team will advance understanding of the mechanisms underlying the cardiac SIRT1 signaling cascade in response to smoking-induced pathological stress. This grant seeks the potential to discover new therapeutic strategies to rescue cardiovascular impairment caused by cigarette smoking exposure. The toxicological constituents of cigarette smoke, including nicotine, carbon monoxide, particulates, oxidants, and heavy metals, indicate the potential to cause systemic injury. Moreover, smoking-induced cardiovascular disease is one major leading to systemic injury in human health. This grant aims to understand how impaired systemic signaling causes a higher incidence of cardiovascular insult in the hypertension population and can discover new therapeutic strategies to limit systemic injury by cigarette smoking in these patients.

Follow on Funding: National Institutes of Health, Ji Li, PhD, 7/1/22-6/30/23, R01 Supplement, Funded: $250,000

Collaborations: 2022.5 - 2022.8, Zehui Li (Junior), Undergraduate Student, Department of Biomedical Engineering, College of Engineering, University of South Florida

2022.5 - 2022.8, Parth Kulkarni (Junior), Undergraduate Student, Department of Biomedical Engineering, College of Engineering, University of South Florida.

2021.5 - present, Migdalia Iglesias (Senior), Undergraduate Student, Biomedical Sciences, University of South Florida.

2021 - present, Linda Ines Zoungrana, PhD candidate, Department of Molecular Pharmacology and Physiology, Morsani College of Medicine, University of South Florida.

2022.1 - present, Hao Wang, Postdoc Fellow, Department of Surgery, Morsani College of Medicine, University of South Florida.

Journals: Zoungrana LI, Krause-Hauch M, Wang H, Fatmi MK, Bates L, Li Z, Kulkami P, Ren D, Li J. (2022) The interaction of mTOR and Nrf2 in neurogenesis and its implication in neurodegenerative diseases. Cells 11: 2048. doi:10.3390/cells11132048. PMID: 35805130.

Patents: None at the time of reporting.

9. **Grant #:** 22K09 Mechanism of Neurotropism by Coronaviruses

Principal Investigator: Mohapatra Subhra, PhD

Organization: University of South Florida

Abstract: It is currently not known how the coronaviruses interact with the glial and endothelial cells of the brain, and this represents an integral hole in understanding about SARS-CoV-2. The blood-brain barrier (BBB) is comprised of endothelial cells, pericytes, and astrocytes. The BBB functions as the primary interface for exchange of nutrients required for brain function while

preventing entrance of toxic molecules. Clinical evidence points to SARS-CoV-2 exploiting the BBB machinery to initiate infection of the brain. This project aims to understand how the virus interacts with the multiple cells of the BBB, if the cells can be directly infected, and molecular machinery responsible for neuroinvasion. The research team found that SARS-CoV-2 is detected in all cells by qPCR. ACE2/Spike protein expression colocalization occurs in human umbilibal vein endothelial cells (HUVEC) infections and DPP4/Spike colocalizes in astrocytes and pericytes. In the transwell BBB model, presence of virus is seen in the upper chamber of the transwell. Viral staining colocalizes the virus with ACE2. SARS-CoV-2 infection does not have any effect on the permeability of fluorescent dextran, demonstrating tight junctions are not affected by infection. Upon SARS-CoV-2 infection, the virus modulates the expression of its respective receptor in HUVECs (ACE2 ↑), astrocytes and pericytes (DPP4↓).

To verify the role of ACE2 and DPP4 in infecting HUVEC, pericytes and astrocytes, the research team has established ACE2- and DPP4-knockout cell lines. The research team is in the process of evaluating the potential of CoV-2 virus infecting the knockout cells compared to wildtype 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.

James and Esther King Biomedical Research Program
Appendix J
Fiscal Year 2021-2022 Active Grants
Funded Fiscal Year 2020-2021

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
21K02	University of Miami	Robert Starke, MD	$535,840.00	4/30/24	No	No	No
21K03	University of Florida	Daiqing Liao, PhD	$535,840.00	4/30/24	Yes	No	Yes
21K04	H. Lee Moffitt Cancer Center and Research Institute	Christine Chung, MD	$1,339,540.00	4/30/26	Yes	No	No
21K05	University of Miami	Carlos Moraes, PhD	$535,840.00	4/30/24	No	Yes	No
21K06	University of Miami	Helen M. Bramlett, PhD	$535,840.00	4/30/24	Yes	Yes	No
21K07	University of Miami	Scott M. Welford, PhD	$535,840.00	4/30/24	No	No	No
21K08	University of Florida	Michelle Gumz, PhD	$535,840.00	4/30/24	No	No	No
21K09	Florida International University	Hoshang Unwalla, PhD	$535,680.00	6/30/24	No	No	No
21K10	Relinquished						
21K11	University of Florida	Chengguo Xing, PhD	$1,114,480.00	4/30/26	No	No	No
21K12	Florida State University	Michelle S. Parvatiyar, PhD	$535,396.00	6/30/24	No	Yes	No
21K13	University of Miami	Adam Wanner, MD	$600,000.00	6/30/24	No	No	No

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

Principal Investigator: Robert Starke, MD

Organization: University of Miami

Abstract: Cerebral aneurysms (CA) are a vascular disorder in which weakening of the arteries in the brain causes localized dilation or bulging of the blood vessel wall. If left untreated CA may rupture resulting in a type of stroke. Clinical data demonstrates cigarette smoking (CS) enhances the risk of aneurysm formation, rupture, and treatment failure. However, it is unclear why CS increases CA risk. Therefore, the purpose of this study is to determine the effects of CS on endothelial cell function during CA formation, progression, and rupture.

Using an experimental aneurysm model, CS exposure induces a change in endothelial cell gene expression resulting in increased expression of inflammation, extracellular matrix remodeling, and stress response genes. The results also demonstrated a reduction in Bnip3 gene expression. This is interesting because Bnip3 is a negative regulator of a protein signaling pathway called mTOR complex 1. Therefore, the research group examined mTOR complex 1 signaling and found a marked reduction in this protein's activity. Consistent with this finding there was an increase in cellular degradation proteins, which are normally inhibited by mTOR complex 1. These data suggest that CS may inhibit mTOR signaling leading to cellular stress and possibly a change in gene expression.

For this study, the research group is also collecting human CA tissue and blood during aneurysm repair surgery and are also using a novel endovascular biopsy technique to collect endothelial cells from within the CA. The specific objective of this part of the study is to use the blood and tissue samples and coil biopsies to determine changes in endothelial cell gene expression, mTOR activation and NOX expression and how this relates to endothelial dysfunction and cerebral aneurysm pathology based upon the patient's smoking status. During this reporting period, the research group has collected two CA tissues, 19 CA blood samples, and three endothelial biopsies, and their corresponding control samples.

The positive impact of this study for Floridians is to provide a foundation for the design of clinical trials to test novel pharmacological inhibitors of CA progression and rupture and minimally invasive CA treatments through endovascular delivery of anti-inflammatory medications which may reduce the enhanced risk of CA rupture in Floridians which 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.

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

Principal Investigator: Daiqing 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 approximately 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 three-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. Increased levels of HDACs in breast cancer correlate with treatment resistance and shortened patient 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 principle investigator's lab has discovered novel HDAC inhibitors that not only inhibit HDAC’s enzymatic activity, but also degrade key components of HDAC enzyme complex. Drugs with such dual mechanisms of action have not been 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. The new HDAC inhibitors are small-molecule compounds and are thus suitable for various systemic treatments, such as via oral administration. Progress has been made towards specific goals of this project. Research outcomes in this reporting period include a new international patent application. This project also supports the training of next generation of scientists and physicians. Importantly, it is anticipated the novel HDAC inhibitors to be tested in

this project may lead to an effective therapy to increase the survival of patients with advanced breast cancer.

Follow on Funding: National Cancer Institute, National Institutes of Health, Daiqing Liao, 4/1/2022-3/31/2027, $3,396,730.00

Collaborations: Nikee Awasthee: PhD, postdoctoral associate (Daiqing Liao, Mentor), University of Florida and Seth Hale: PhD graduate student (Daiqing Liao, mentor), University of Florida

Journals: None at the time of reporting.

Patents: International Patent Application, Serial No. PCT/US22/73187, filed June 27, 2022 Title: Compounds and Methods of Use for Degrading Rest Compressor 1, Lysine-Specific Histone Demethylase, Histone Deacetylase 1 and Histone Deacetylase 2 in the Corest Comple

3. **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 and Research Institute

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 such as pembrolizumab which inhibits programmed cell death-1 (PD-1) has become a promising therapeutic option in HNSCC. 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. The research team determined the tumor microenvironment of tobacco-related HNSCC is immunosuppressive, in part due to hypoxia which upregulates vascular endothelial growth factor (VEGF). Recent data support the immunosuppressive roles of VEGF. While preclinical and clinical evidence of anti-angiogenesis as an effective therapeutic option for HNSCC patients is existing, the team proposed to evaluate an anti-angiogenic agent, cabozantinib, in context of immunotherapy in hypoxic HNSCC. During this legislative period, the research team completed a phase II multicenter single arm trial of pembrolizumab and cabozantininb in patients with R/M HNSCC. A total of 50 patients were screened and 36 were enrolled with 33 evaluable for response. Seventeen out of 33 (52%) patients had a partial response and 13 (39%) had stable disease with an overall clinical benefit of 91%. Median and 1-year overall survival were 22.3 months (95% CI: 11.7-32.9) and 68.4% (95% CI: 45.1-83.5), respectively. Median and 1-year 37 progression free survival were 14.6 months (95% CI: 8.2-19.6) and 54% (95% CI: 31.5-38 72), respectively. Pembrolizumab and cabozantinib was well tolerated and showed very promising clinical activity in patients with R/M HNSCC. Frequent grade 3 or higher treatment related adverse events were increased aspartate transaminase (AST) and hyponatremia (n=3, 8.3%). Seventeen patients (47.2%) had dose reduction of cabozantinib to 20 mg daily. With the promising results, further clinical development of this regimen is expected to move forward. The researchers are currently generating

laboratory data to further evaluate the mechanism of potential treatment resistance and to identify predictive biomarkers of the response. According to data from Centers for Disease Control and Prevention (CDC), 16.1% of adults smoked, and 2.7% of adults used smokeless tobacco in Florida in 2017. The findings from this project will have an immediate impact in the health of Floridians by improving the efficacy of existing treatment options as well as development of novel therapies for Floridians with tobacco related HNSCC. In addition, this research will improve the clinical utility and economic value of PD-1 inhibitor containing regimens by developing predictive biomarkers of clinical benefits. Using these agents in only selected patients who will benefit the most will have a significant impact in the health care cost and quality of care.

Follow on Funding: Grateful patient donation to develop new immunotherapies for oral cancer, Christine Chung, MD, 7/20/2022-no end date, Marshall Glenn Hoffman Immunotherapry Fund for Oral cancer Research, FUNDED: $5 million

Collaborations: Emory University, Atlanta GA: Academic collaboration through participating in the cabozantinib and pembrolizumab clinical trial. The biospecimens collected at Emory from the trial are being sent to Moffitt for biomarker analyses.
Broad Institute of MIT

Journals: None at the time of reporting.

Patents: None at the time of reporting.

4. **Grant #:** 21K05 Mechanisms of mitochondrial DNA deletion formation

Principal Investigator: Carlos Moraes, PhD

Organization: University of Miami

Abstract: The research team continued to breed and analyze Mgme1 knockout, mitoPstI transgenic and SOD2 +/-. Researchers have started to smoke the SOD2+/- crosses and have implemented a new approach to look for mtDNA deletions. Namely, digital PCR, which is more precise than qPCR in detecting mtDNA deletions. Using digital PCR (dPCR) staff can detect simultaneously the partially deleted mtDNA, a reference region of mtDNA and nuclear DNA. As exemplified in figure 1, researchers believe this approach will allow us to define better the mechanisms of mtDNA deletion accumulation. Research staff have also optimized laser capture microscopy to analyze the levels of mtDNA deletions in different brain regions of mice exposed or not to smoke.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Mitochondrial genome engineering coming-of-age. Barrera-Paez JD, Moraes CT. Trends Genet. 2022 Aug;38(8):869-880. doi: 10.1016

Patents: None at the time of reporting.

5. **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: Stroke is one of the leading causes of disability and death in the United States, and mitigating the causes of stroke is a major public health challenge. Approximately 87% of strokes are caused by a blockage in a cerebral artery, leading to ischemic brain damage. On average, smokers experience a stroke 10 years earlier than nonsmokers. In recent years, people trying to give up smoking are turning to Electronic Cigarettes (EC). ECs work by heating a liquid to produce an aerosol that usually contains nicotine, flavoring compounds, and other chemicals, which are inhaled during vaping. EC aerosols are depicted to contain a lower number and overall quantity of harmful toxicants than conventional cigarettes (CC). However, emerging research indicates that EC aerosols contain harmful ingredients including ultrafine particles, volatile organic compounds, and heavy metals. One common ingredient found in both CC and ECs is nicotine, which has been shown to be both highly addictive and toxic. Particularly relevant to the study, multiple studies published from the laboratory show that smoking-derived nicotine exacerbates ischemic stroke outcomes. Therefore, the question arises; will EC use impact the outcome of stroke? To answer this question researchers established a mouse model of EC exposure. Mice or Rats are exposed to either air or EC vapor (5% nicotine Juul pods) using the EcigAero-TM Aerosol Exposure Apparatus (between 7pm-7am; the active phase of circadian cycle in animals) for 16 nights. Per night, rats were exposed to 16 episodes of EC. Each episode consisted of the two seconds of Juul puffs followed by eight seconds of air over the period of eight minutes. Using this paradigm of EC exposure, the researchers confirmed presence nicotinine levels in the brain and achieved levels similar to that of serum in humans. Additionally, using unbiased global metabolomics approach their recent study showed that EC exposure significantly increased histidine, glutamate, phenylalanine, and tyrosine metabolites in males and females ($p<0.05$). Tryptophan metabolites were significantly increased in the brain of only male animals ($p<0.05$). Based on these results the researchers conclude that chronic nicotine use alters levels of amino acids metabolites in the brain of animals. In parallel, the research teamalso successfully established a photothrombotic mice model of stroke and currently investigating effects of stroke and proposed therapy of whole body vibration in EC exposed animals. Finally, publications and grant support have been generated in part due to funding from Florida Department of Health. The researchers also presented their stroke research at the conferences listed below.

Kerr N, Sanchez J, Moreno WJ, Furones-Alonso OE, Dietrich WD, Bramlett HB and Raval AP. Irisin, elicited by low frequency whole body vibration or exogenously, improves post-stroke cognition and reduces infarct volume in middle-aged rats. International Stroke Conference March 2022-Heald In-person and Virtual at New Orleans February 8 - 11 2022 (Refereed) Sex differences in post-stroke whole body vibration therapy on the cognitive outcomes. The Conference of Organization for the study of sex differences at Marina Del Rey, California between May second and fifth, 2022.

N Kerr, J Sanchez, W Moreno,O Furones-Alonso, W Dietrich,H Bramlett and A Raval. Sex difference in cognitive improvement after post-stroke low frequency whole body vibration therapy in rats. Brain and Brain PET 2022 held in Glasgow.UK 29 May-1 June 2022.(Refereed) Abstract published in JCBFM 22;42(15) 108-273

Follow on Funding: Veterans Affairs, 10/1/22-9/30/26, Merit review,$1,200,000

Collaborations: None at the time of reporting.

Journals: Kerr NA, Sanchez J, O'Connor G, Watson BD, Daunert S, Bramlett HM, Dietrich WD.• Inflammasome-Regulated Pyroptotic Cell Death in Disruption of the Gut-Brain Axis After Stroke.Transl Stroke Res. 2022 Mar 19. doi: 10.1007/s12975-022-01005-8. Online ahead of print.

Patents: None at the time of reporting.

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

Principal Investigator: Scott M. Welford, PhD

Organization: University of Miami

Abstract: Advanced clear cell renal cell carcinoma (ccRCC) leads to death within 5 years for nearly 90% of patients due to poor responses to current therapies; thus new therapeutics are sorely needed. Tobacco smoking and obesity are the two most well-established risk factors for renal cancer incidence, and are linked coincidently to similar general health morbidities (such as hypertension, heart disease, and metabolic syndrome) that suggest the existence of potentially overlapping etiological mechanisms. ccRCC tumors are unique in that the tumors display histologic and genetic signs of adipogenic transdifferentiation, including gross lipid and glycogen-rich cytoplasmic deposits. The research team identified a mechanism driving lipid deposition, and showed that preventing lipid storage restricts tumorigenesis. The data suggest that targeting metabolism holds therapeutic promise. The purpose of the present application is to investigate the role of an adipokine (named Chemerin) that is overexpressed in ccRCC and in obesity, controls lipid metabolism, and is prognostic for outcome. Strikingly, Chemerin is also induced by smoking. Thus Chemerin represents a possible link between smoking, body mass, and renal cancer risk, that has therapeutic implications.

In the first year of the award, the research team has made significant progress towards the aims of the project. For Aim 1, the research team has proposed to build a catalogue of ccRCC tumor samples in the form of tumor microarray (TMA), which is comprised of 90 University of Miami patient tumors and local normal kidney specimens spotted onto three microscope slides. The significance of producing their own TMA is that the patients come from the South Florida catchment area, and have complete medical history and records that will allow the team to determine associations of obesity and smoking with tumor characteristics. The research team has successfully collected the samples via collaboration with Dr. Mark Gonzalgo (urologist), produced the TMA, and stained for several markers. The research team is presently in process of assessing the results. In Aim 2, the team has proposed to use a monoclonal antibody with patient derived xenografts to assess the impact of obesity on tumor growth. Challenges have been making host immunocompromised mice obese, and thus are now changing to a genetically engineered mouse model, in which obesity is more reproducible. The researchers are in the 3rd of four stages to produce the model, and look to design experiments in the next funding year.

Follow on Funding: None at the time of reporting.

Collaborations: University of Miami, Miami FL, USA Cancer Biology Program Students: Sze Kiat Tan Dazhi Wang Melanie Stone

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Michelle Gumz, PhD

Organization: University of Florida

Abstract: Smoking leads to high blood pressures and multiple cardiovascular diseases. The circadian clock, which controls most bodily rhythms, is affected by smoking and is an important regulator of blood pressure. The research team has identified that in the kidney, the circadian protein Period 1 (PER1) modulates Endothelin-1 (ET-1) levels to affect salt re-absorption. 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. The researchers hypothesize that PER1 in endothelial cells modulates systemic vascular resistance and salt-sensitive hypertension. Researchers have recently identified 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. PER1 could be a novel therapeutic target for hypertension. The clinical development of ET-1 antagonists has been limited by side effects. Thus, targeting PER1 or EDN1-AS might be a novel way to antagonize ET-1 in a tissue specific manner for therapeutic purposes.

In the first experiment, male and female PER1 Knock-out (KO) and wild-type (WT) mice were subjected to echocardiographic measurement of pulse wave velocity (PWV) to assess aortic stiffness. It was observed that knockout of PER1 resulted in an increased vascular stiffness in male but not in female mice. Arterial stiffness is an independent predictor of cardiovascular disease in patients with hypertension. It seems that the risk factors involved in the pathology of uncontrolled hypertension are similar to those that contribute to the development of arterial stiffness. Further, cell specific mRNA expression of lysyl oxidase (LOX), an extracellular cuproenzyme that mediates collagen cross-linking, was assessed in aortic tissue using in-situ hybridization (ISH). LOX mRNA expression found to be increased in the aorta of PER1 KO compared to WT mice. LOX is integral to maintain vascular stiffness, and exhibits a circadian pattern of expression. Taken all together, the findings suggest that disruption of circadian rhythm by deleting PER1 increases arterial stiffness in male but not in female mice. Increased LOX mediated changes in extracellular matrix are at least in part responsible for the pathogenesis of arterial stiffness in these mice.

In the second experiment, the researchers collected the aorta samples from a large cohort of mice (4 groups: 1) WT control diet, 2) WT high salt diet plus DOCP, 3) PER1 KO control diet, and 4) PER1 KO high salt diet plus DOCP; n=5 per group). The research team performed and quantified ISH experiments to determine cell-specific expression of ET1 mRNA in PER1 KO and WT mice. The researchers have established two new breeding mouse colonies with endothelial and vascular smooth muscle cells (VSMCs) specific deletion of PER1. There are now sufficient number of mice for the experiments aimed to study the role of vascular PER1 in systemic vascular resistance and salt sensitive hypertension.

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 #:** 21K09 Pathophysiological Mechanisms and Therapeutics for Chronic Lung Inflammation in Smokers and in COPD

Principal Investigator: Hoshang Unwalla, PhD

Organization: Florida International University

Abstract: The long-term goal of this proposal is to arrest or reverse lung inflammation in chronic obstructive pulmonary disease (COPD). Cigarette smoking is the primary cause of COPD. Chronic inflammation is a hallmark of COPD. Mitochondrial dysfunction due to the inability of airway epithelial cells to eliminate aged mitochondria (impaired mitophagy) promotes senescence leading to a senescence associated secretory phenotype and secretion of proinflammatory cytokines. Cystic fibrosis transmembrane conductance regulator (CFTR) along with airway lactoperoxidase (LPO) regulates hydrogen peroxide levels in the airway. Cigarette smoke suppresses CFTR and LPO by inducing small RNA molecules called microRNAs leading to increased hydrogen peroxide in the airway. This promotes impaired mitophagy and senescence leading to lung inflammation in COPD. Identifying these mechanisms and developing therapies to boost CFTR and LPO can restore normal hydrogen peroxide levels and prevent lung inflammation in COPD.

Progress to date has demonstrated:
1) Publication that Transforming growth factor-beta (TGF-beta) is induced by cigarette smoke, TGF-beta suppresses LPO protein in BEAS2B airway epithelial cell lines.

2) TGF-beta increases expression of the microRNA miR-449b. The researchers had hypothesized that miR-449b would bind to LPO mRNA and suppress it based on the microRNA scores (miRSVR score).

3) TGF-beta, which is induced by cigarette smoke suppresses LPO protein in 3D model of bronchial epithelium (bronchial epithelium redifferentiated at the air-liquid interface).

4) TGF-beta increases hydrogen peroxide levels in the team's 3D model of bronchial epithelium.

5) The preliminary data demonstrated that TGF-beta and hydrogen peroxide impair mitophagy and promote senescence. The researchers demonstrate that miR-449b suppresses LPO. It was demonstrated by using synthetic miR-449b (miR-449b mimic) in the 3D model of bronchial epithelium as well as by using an inhibitor of miR-449b to show restoration of LPO levels. Together these data confirm their hypothesis that TGF-beta suppresses LPO by inducing airway epithelial cells to produce more miR-449b.

6) The researchers show that like TGF-beta, miR-449b mimic also impairs mitophagy and promotes senescence in airway epithelial cells.

7) Finally, it was demonstrated that TGF-beta and its downstream effects lead to changes in the mitochondrial membrane in 3D model of airway epithelium leading to its depolarization as the first step towards impaired mitophagy, senescence and lung inflammation.

Together the research team has made sufficient progress towards completing Subaim1 of Aim1. which is to determine the mechanism by which cigarette smoke promotes inflammation. Impact to Floridians: Tobacco smoke is the main cause of COPD. In 2020 COPD accounted for around 30,000 hospitalizations in Florida leading to a significant economic burden and lost wages for the state as well as caregivers. This research will identify the mechanisms by which cigarette smoke promotes chronic inflammation in the lung and test therapeutic approaches to mitigate this inflammation. The researchers anticipate that the outcomes will improve the quality of life of Floridians living with COPD.

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 #:** 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: As proposed, the goal of this grant was to evaluate the potential of flavokavains A and B (AB)-free kava in reducing lung cancer risks among active smokers with no intention to quit via a double-blind randomized placebo controlled trial. During the past funding period, the research team has successfully obtained Investigational New Drug (IND) approval from Food and Drug Administration (157256). The research team also had the clinical protocols reviewed by a series of review panels at University of Florida and obtained final Institutional Review Board (IRB) approval (IRB202101885). The research team has obtained the investigational kava capsules and the placebo capsules from Thorne and established the dispensing protocols with Investigational Drug Service at University of Florida. The research team has also established an agreement with the clinical labs at Shands Hospital for clinical chemistry analysis. An independent Research Electronic Data Capture (REDCAP) database has been established to organize the data and keep the record of the clinical process. Finally the active recruitment was initiated. Along these processes, the protocols have been optimized with IRB revisions implemented on a timely manner. To facilitate enrollment, the research team has implemented various activities, including flyers and presentations. The team continues to meet regularly to optimize the clinical protocol, to identify potential gaps, and address issues. The research team is also developing a protocol manuscript. In summary, adequate progress has been made to this grant during the past funding period with active recruitment ongoing, which will be the main goal of the next funding period.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Michelle S. Parvatiyar, PhD

Organization: Florida State University

Abstract: Scientific accomplishments by research staff this year include obtaining approvals from animal use committees, institutional human research board, state, and federal agencies. The purpose of this study is to examine the origins of sustained inflammation in overweight individuals especially in the context of smokers versus non-smokers. Mouse models are being used to understand how obesity and exposure to cigarette smoke increase pro-inflammatory pathways. Approvals were obtained by project staff to collect blood from patients who are obese and/or smokers to correlate these findings to the animal studies.

Progress on this project includes studies that examine the role of the gene of interest sarcospan in regulating the immune response. Studies have been performed in non-smoking controls to obtain data that will be compared to smoke exposed mice. Since many conditions are being compared: sex, gene background, obese versus nonobese, smoking versus non-smoking – it is important to examine these different conditions in discrete blocks to ease execution of the project. Removal of the gene sarcospan protects both young and middle-aged male and female mice from obesity and insulin resistance – with the strongest influence in females. While these are favorable outcomes, there is also increased activation of immune markers in the blood of sarcospan deleted mice and it is still unclear what this impact will be in smokers. Further investigation is required to understand the impact of immune cell activation in these mice. In tissues from smoked mice the amount of sarcospan expressed in the lungs and hearts of mice is dramatically decreased which could increase the severity of lung and heart injury in smokers. In the next studies both male and female mice will be examined with and without sarcospan to determine the time course of systemic and tissue inflammation due to smoking. Research staff have submitted two peer-reviewed abstracts to international meetings, one review paper is under review related to this project and two papers will be submitted in the coming months.

Impact to Floridians (health and return on investment): This study will provide information on whether compounds that modulate sarcospan expression can protect smokers from immune activation and obesity that can cause damage heart tissue. The studies conducted by research staff in mice will be compared to human studies examining obese individuals and/or smokers by examining inflammatory markers that occur in each condition with the amount of immune cell sarcospan expression.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Crawford RQ, Valera IC, Pindado J, Reis G, Rahimi Kahmini A, Mumbi F, Parvatiyar K and Parvatiyar MS (2022) Sarcospan-deficient mice exhibit a heightened inflammatory phenotype under obesiogenic conditions. FASEB J 13 May 2022. https://doi.org/10.1096/fasebj.2022.36.S1.R6079

Patents: None at the time of reporting.

11. **Grant #:** 21K13 Early Detection of Vaping-related Vascular Disease

Principal Investigator: Adam Wanner, MD

Organization: University of Miami

Abstract: It has been well established that cigarette smoking leads to vascular disease including coronary heart disease and stroke. The toxins contained in tobacco smoke damage the inner layer of blood vessels (endothelium), leading to endothelial dysfunction (blunted dilation of blood vessels). It is not known if e-cigarette use (vaping) has the same effect. The research team reasoned that the blood vessels in the lung would be exposed to the highest toxin levels contained in the inhaled vapor and that the earliest site of endothelial dysfunction would be seen in the lung vessels. This premise forms the basis of this 3-year grant. The overall objective is to assess vascular endothelial function in the lung and forearm of habitual vapers relative to non-vapers thereby testing the hypothesis that the lung vessels are more sensitive in capturing early vascular toxicity.

In aim one, the research team set out to enroll 60 young vapers and 30 non-vapers without a history of cigarette smoking to measure endothelial function non-invasively in-and outside the lung. There have been delays in participant enrollment for technical reasong (COVID pandemic-related delivery issues with a mass-spectrometer and the difficulty of securing the test gas mixture needed for the measurements). However, enrollment is still on schedule to complete full final enrollment over the next two years, starting in August 2022. Aim two is to determine the toxic effect of vape condensate on endothelial cells in culture in an attempt to clarify the mechanism of vape induced vascular toxicity. To date, the endothelial cell culture experiments have already shown that e-cigarette vapor condensate induces dose-dependent increases in the generation of toxic oxygen radicals, with variation among different e-cigarette brands.

The plan for the coming year is to enroll 66% of participants and to study how oxidants interfere with the expression and function of proteins involved in endothelium-dependent vasodilation. Initial results will be presented at the next annual International Conference of the American Thoracic Society in May 2023, with a publication in a refereed journal in the same year. The researchers do not expect any reportable data regarding aim one before 2024 (after full participant enrollment and statistical analysis).

Given the high prevalence of de-novo electronic cigarette vaping among the young in Florida, the researchers believe that demonstrating early vascular injury in this population will be critical for educational purposes and for tracking potential vascular risk.

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 K
Fiscal Year 2021-2022 Active Grants
Funded Fiscal Year 2019-2020

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
20K01	Florida State University	Pradeep G. Bhide, PhD	$626,708.00	6/10/23	No	Yes	No
20K02	Mayo Clinic Jacksonville	Debabrata Mukhopadhyay, PhD	$626,708.00	4/30/23	No	No	No
20K04	University of Central Florida	Ulas Bagci, PhD	$1,112,880.00	4/30/23	No	Yes	No
20K05	University of Florida	Terence Ryan, PhD	$626,710.00	5/31/23	No	No	No
20K06	University of Florida	Gilbert Upchurch, Jr., MD	$626,708.00	5/31/23	No	No	No
20K07	University of Florida	Daiqing Liao, PhD	$626,708.00	5/31/23	No	No	No
20K08	University of Florida	Dorian K. Roase, PhD, MS, PT	$688,940.00	4/30/25	No	No	No
20K09	University of Miami	Ami Raval, PhD, MSPH	$626,710.00	5/31/23	Yes	Yes	No
20K10	University of Miami	Taghrid Asfar, MD, MSPH	$1,253,415.00	6/30/22	No	No	No
20K11	University of Miami	Miguel Perez-Pinzon, PhD	$626,708.00	6/30/23	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 combustible and e-cigarette exposures on the developing brain, germ cells and future generations using a mouse model. The initial studies focused on exposure of mice to combustible cigarette smoke. During the current reporting period, e-cigarette or e-liquid aerosol exposure was examined. Adult female mice were exposed to e-cigarette aerosol, e-liquid aerosol, or room air (control) for 58 min per day. The exposure began three-four weeks prior to breeding the mice with drug naïve males and continued throughout pregnancy. Upon parturition, the mother and her offspring were exposed for another three weeks. Thus, the developing brain was exposed in the prenatal and early postnatal (i.e., pre-weaning) periods. This exposure paradigm corresponds to human exposures beginning prior to conception and continuing through all three trimesters of pregnancy. The health and wellbeing of the mice were monitored daily by visual inspection. In addition, the mice were weighed at the beginning of the exposure as well as twice a week throughout the exposure period. There was no statistically significant difference in the percentage change in body weight between the room air (control) group and the either of the two aerosol exposure groups. Moreover, the offspring in the three groups (e-cigarette, e-liquid and room air) acquired developmental milestones approximately at the same time.

However, these seemingly normal developmental events obscured the significant changes that were occurring in the brain. When the mice reached two to three months of age, behavioral studies were performed. It was found the mice that were exposed to e-cigarette aerosol in the prenatal and early postnatal periods showed significant risk-taking behavior and motor impulsivity compared to their counterparts exposed to e-liquid aerosol or room air during the prenatal and early postnatal periods. It has been reported that these behaviors are associated with significant increase in drug addiction risk, although the researchers did not analyze this risk in these studies. Thus, exposure of the developing brain to e-cigarette aerosol produced significant behavioral changes that appeared to be long-lasting because the chaanges were evident at three months of age.

In summary, these data show that electronic cigarette aerosol exposure in the prenatal and early postnatal periods produces behavioral impairments that last well into adulthood. Therefore, e-cigarette use by women during pregnancy and nursing periods may produce lasting adverse impacts on the mental health of the children.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Zhang L, McCarthy DM, Eskow Jaunarajs KL, Biederman J, Spencer TJ, Bhide PG. Frontal Cortical Monoamine Release, Attention, and Working Memory in a Perinatal Nicotine Exposure Mouse Model Following Kappa Opioid Receptor Antagonism. Cereb Cortex. 2021;3

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: Purpose: 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. Context: Smoking is not only the primary risk factor of lung cancer, but it also increases drug resistance by activating alternative signaling pathways. Concurrent inhibition of these alternative pathways may override drug-resistance and improve prognosis in lung cancer patients, especially those with a smoking history.

Progress: During the past year, the following was accomplished:

i) A new dexamethasone containing liposomal formulation of NDT-19 (D1XN19) was developed to overcome NDT19-mediated induction of inflammatory cytokines. The in vivo efficacy of D1X19 was assessed in orthtopic LLC1 tumors. Although D1XN19 treated group showed slight improvement in median survival, no significant benefit was observed in tumor growth inhibition.

ii) To identify a better inhibitor, several new NDT-19 analogs were tested in LLC1 for in vitro cytokine expression. However, all of these analogs showed increased expression of various inflammatory cytokines as was observed in case of NDT-19. Therefore, the research staff abandoned NDT-19 and focused of #9 for further studies.

iii) The in vitro efficacy of #9, a novel NRP-1 inhibitor, was analyzed in two Epidermal Growth Factor Receptor (EGFR)-mutant non-small cell lung cancer cell lines (H1650, H1975) and compared with cabozantinib. #9 was slightly more effective in H1975 cell line compared to cabozantinib but was less effective in H1650 cell lines.

iv) The in vivo efficacy of #9 was assessed in H1650 and H1975 tumors and compared with Cabozantinib. Both #9 and Cabozantinib inhibited tumor growth in these xenografts, but the combination did not show significant improvement over monotherapies.

v) The efficacy of #9 in overriding resistance to Erlotinib, a first generation EGFR tyrosine kinase inhibitor (TKI), was evaluated in subcutaneous H1650 tumors. The combination of #9 with Erlotinib showed remarkable tumor growth inhibition than Erlotinib monotherapy in this experiment.

vi) The efficacy of #9, either alone or in combination with anti-Programmed Death-1 (PD-1) antibody, was evaluated in orthotopic LLC1 tumors. Although #9 or Anti-PD-1 antibody did not improve the survival in this experiment, surprisingly the combination showed a significant improvement in survival. This experiment was repeated once more to validate the reproducibility. A similar trend was observed, however the increase in median survival of the combination over anti-PD-1 antibody monotherapy was less spectacular this time. Still, it is now clear that #9 has a consistent and significant synergistic effect on anti-PD-1 therapy.

vii) To delineate how #9 is showing synergism with anti-PD-1 therapy, alterations in various cytokines was analyzed in LLC1 cells treated with increasing concentrations of #9. Increase in various immunomodulatory cytokines was observed in this experiment, further studies are in progress.

Impact: 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: The overall goal of this research project is to develop Artificial Intelligence (AI) based image analysis tools to assist clinicians during the radiotherapy treatment phase of lung cancer patients. When a patient has a cancerous region(s) in his or her lung and is treated using radiotherapy, the clinician performs an initial treatment and requests follow-up imaging scans to watch the progression of the healing process. In some cases the cancer recurs, but there can be a delay before the recurrent cancer is visible on medical imaging scans. The tools being developed in this project will assist clinicians to detect any recurrence earlier from medical scans by detecting very subtle changes in the imagery through the use of trained deep neural networks (DNNs). Furthermore, the research team plans to develop AI solutions that can predict cancer recurrence even before it happens, based on a retrospective analysis of the conditions, treatments, and results from past patients treated at the partner hospital, Orlando Health.

During this past year, the research team developed the core AI models to automatically detect the boundaries of lungs and cancerous lung lesions directly (without human assistance) from a patient's medical imaging scan (CT scans). These tools will save clinician time and improve consistency in interpreting patient imaging scans. Additionally, the team trained a neural network model on imagery shared by Orlando Health to predict the two-year survival outcome of a lung cancer patient using only their initial chest imaging scan as input. Some patients were set aside that the model had never seen before as a test set and the model predicted the survival correctly for 88% of these test cases! The research team expects to be able to further improve this accuracy by adding additional input data into later versions of this model. In addition, an organ at risk (OAR) segmentation method has been developed that is useful for prescribing the radiation dose in the treatment phase where the aim is to minimize the dose to organs while maximize it to cancer tissue.

One way to collect additional data from the retrospective set of patients and improve future AI models, is to have expert radiologists create what are called "annotations" for these images. In this context, an image annotation is a characteristic that is observed as either present or not present in an image. Together, these annotations form a useful description that accompanies the pixels of each image and can be used to train AI models. Generally annotations are hard and time-consuming to collect. During this year, the team created a web-based annotation system that displays a patient's images and automatically records annotations select by clinicians for each patient's image. The web-based annotation system is hosted in the Amazon Cloud and can be re-used to collect annotations for many different types of images. This tool is crucial in the project, but it is also usable to annotate images for training AI models to detect other types of cancer, beyond the lung cancer currently focused on.

The project is developing automatic image analysis tools that will help clinicians in Florida more quickly to make radiation dose calculation optimal, and then estimate the length of survival for lung cancer patients and also more quickly detect if or when cancer will return after radiotherapy treatment.

Follow on Funding: None at the time of reporting.

Collaborations: This project is supporting the Doctoral Thesis work of Ilkin Isler, a Doctoral Candidate in the School of Computer Science at the University of Central Florida, Orlando, FL

Journals: Isler, I., Lisle, C., Rineer, J., Kelly, P., Turgut, D., Ricci, J., Bagci, U.,, "Enhancing organ at risk segmentation with improved deep neural networks", SPIE Medical Imaging Symposium, 20-24 February 2022, San Diego, California, USA. Srivastava, A., J.

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 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 state-wide. 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.

In this reporting period, significant progress has been made toward the completion of the experiments proposed in this award. Some data have been unblinded to the principal investigator while research staff remain blinded to ensure the most rigorous processes are maintained for scientific integrity. Major accomplishments and discoveries are discussed below:

1) The researchers are the first to discover a role of the aryl hydrocarbon receptor (AHR) in regulating skeletal muscle metabolism. To date, the AHR has been almost exclusively studied in the liver or immune system. The researchers' studies have revealed that removal of the AHR from skeletal muscle can protect the muscle metabolic health in mice that underwent chronic cigarette smoking. This finding even held for mice in middle-late age. This is an important finding because people that are chronic smokers commonly report symptoms of fatigue and exercise intolerance which are symptoms heavily linked to metabolic problems in muscle. More importantly, the discovery provides a foundation for testing inhibitors of the AHR in past or current smokers with the hopes of improving their quality of life and muscle function. Related to this, several AHR inhibitors are currently being tested in patients for other diseases.

2) Consistent with the improvements discussed in #1 above, the team found that expression of a mutant AHR that is always active also disrupts muscle metabolism and mimics the effects observed in mice with the AHR exposed to cigarette smoke. This is also a discovery that has not been reported previously. Genomic analyses were performed to try and understand the pathways altered in muscle by AHR activation. These data are current being analyzed and plan to leverage that data toward future grant submissions.

3) Another major accomplishment in this reporting period was the publication in the journal TOXICS (Khattri RB, Thome T, Fitzgerald LF, Wohlgemuth SE, Hepple RT, Ryan TE*. NMR spectroscopy identifies chemicals in cigarette smoke condensate that impair skeletal muscle mitochondrial function. Toxics 10(3): 140, 2022. https://doi.org/10.3390/toxics10030140) that described the approach to analyzing chemical components in cigarette smoke that negatively impact muscle mitochondrial metabolism.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Khattri RB, Thome T, Fitzgerald LF, Wohlgemuth SE, Hepple RT, Ryan TE*. NMR spectroscopy identifies chemicals in cigarette smoke condensate that impair skeletal muscle mitochondrial function. Toxics 10(3): 140, 2022. https://doi.org/10.3390/toxics10030140

Patents: None at the time of reporting.

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

Principal Investigator: Gilbert Upchurch, Jr., MD

Organization: University of Florida

Abstract: Purpose: To investigate the effect of nicotine on immune cell infiltration and activation of myeloid-derived suppressor cells (MDSCs) via chemokine receptor type 2 (CXCR2) and Interleukin-17 (IL-17) signaling that mediates abdominal aortic aneurysm (AAA) formation.

Context: Aortic aneurysms affect five percent 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, the researchers 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, analysis will be 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 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.

Progress to date: New data shows no significant difference between elastase-treated WT mice exposed to smoking compared to elastase-treated mice alone (177.6 ±8.6 vs. 175.3±8.3; n=14/group; p=0.98). Recent data showed a trend towards decrease in M-MDSCs in elastase-treated cebpb null mice on day 14 compared to elastase-treated balb/c mice (0.3±0.1% vs. 0.5±0.1%; n=3-4 mice/group). Similarly, a decrease in G-MDSCs was observed in elastase-treated cebpb null mice compared to elastase-treated balb/c mice (1.2±0.4 vs. 2.3±0.9%; n=3-4 mice/group). Previously it was observed that elastase-treatment of 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. 0.4±0.1%; mean±S.E; p=0.02; n=4/group).

The in vitro data shows that the secretion of IL-1 from RAW264.7 macrophages was significantly enhanced by concomitant nicotine treatment (10M) 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 the mean ± standard error of the mean (SEM). One-way ANOVA after Tukey's statistical test was used to determine the differences among multiple groups and Mann-Whitney test was used for pair-wise comparisons of groups. A value of $p<0.05$ was considered statistically significant.

Impact to Floridians: 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 the recent results. The 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: None at the time of reporting.

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 diagnosed cancer type and the second leading cause of cancer-related mortality for women. About 1 in 8 women in the U.S. will develop invasive breast cancer over the course of her lifetime. In 2022, 290,560 new cases of breast cancer are expected to be diagnosed in the U.S. with 20,920 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 their proliferation and drives cancer progression. The goal of this grant is to determine the mechanisms underlying increased lipid production in breast cancer cells and test potential therapy to inhibit this process as a safe and effective treatment for patients with advanced breast cancer. This grant has enabled the research team at University of Florida to advance the field of breast cancer biology with peer-reviewed publications, training of future scientists and physicians at undergraduate and graduate level, and the creation of intellectual properties. The new scientific discovery from this project also supported federal grant applications. 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: Iqbal Mahmud: Ph.D. postdoctoral associate (Daiqing Liao, Mentor), University of Florida Aaron Waddell: Ph.D. graduate (Daiqing Liao, mentor), University of Florida Chengcheng Meng: Ph.D. graduate student (Daiqing Liao, mentor), University of Florida

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Dorian K. Roase, PhD, MS, PT

Organization: University of Florida

Abstract: Thirty-five community-dwelling individuals post-stroke have been enrollled in this randomized controlled trial. As the trial is ongoing, researchers do not have definitive results to report at this time. Data from this grant was presented at the American Physical Therapy Association's Combined Sections Meeting, February 4, 2022 in San Antonio, Texas, "A Physical Therapist-Led Community Exercise Program to Counter Post-Stroke Fatigue." The poster was recipient of the Blue Ribbon Award from the Health Policy Administration Section/Global Health Special Interest Group in recognition of serving underserved populations. All enrolled study participants have completed the intended exercise intervention, tolerating the intervention well.

The majority of participants have chosen to continue to exercise to reduce their risk for secondary stroke, once their study participation has completed. The researchers continue to make progress towards all three Specific Aims.

Follow on Funding: None at the time of reporting.

Collaborations: University of Florida/College of Public Health and Health Professions/Department of Physical Therapy: The study's PI and Co-PI are located here. A PhD student in the Rehabilitation Science program is assisting with data management. University of Florida/College of Health and Human Performance/Department of Applied Physiology and Kinesiology: Four undergraduate interns assisted in data management and assisting with the study intervention. University of Florida/College of Engineering/Dept. of Biomedical Engineering: One undergraduate student has participated in data management. University of North Florida/Brooks College of Health/Department of Physical Therapy: Two Doctor of Physical Therapy students three undergraduate interns have assisted with data management and study intervention.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Ami Raval, PhD, MSPH

Organization: University of Miami

Abstract: Stroke disproportionately kills more women than men and is one of the leading causes of death and disability worldwide. Women have both an increased lifetime risk for stroke and a higher mortality rate than men, and recent epidemiological data show that young women suffer more strokes than young men. The major preventable risk factor for stroke is cigarette smoking and especially in the era of electronic cigarettes, it remains a top concern. The common agent found in conventional or electronic cigarettes is nicotine. In a study using a rat model of ischemia, the researchers demonstrated that nicotine (N) induces changes in the metabolism of histamine, leading to severe hypo-perfusion thus exacerbating ischemic brain damage in young female rats. The goal of the proposed Aim 2 is to investigate how long this nicotine toxicity persists in the brain after nicotine withdrawal (NW). Additionally, post-ischemic cognitive decline is a significant consequence, and the researchers aim to test how ischemic injury after nicotine exposure or NW affects cognitive decline. In the current study, adult female Sprague-Dawley rats (n=8/group) were randomly exposed to either saline or N (4.5 mg/kg) for 16-21 days. Followed by the withdrawal of nicotine exposure, the rats were allowed to recover for 0-, 15-, or 30-days. After completion of the assigned withdrawal period, the rats were randomly assigned to receive either a transient middle-cerebral artery occlusion (tMCAO; 90 min), sham surgery, or have their brain tissue collected for global metabolomic (Metabolon Inc) and western blot analysis. One-month post-surgery, the rats were tested for hippocampal-dependent contextual fear conditioning where % freeze time was measured. Following this behavioral testing, brain tissue was harvested for quantification of infarction. Upon completion of the treatment and subsequent withdrawal period, the infarct volume was quantified to be 26% ($p<0.05$), 25% ($p<0.05$), and 16% ($p<0.05$) higher in the 0-, 15-, and 30-day NW groups respectively, compared to the saline group. The recorded fear conditioning data demonstrated significantly lower freezing in all three NW groups as compared to the saline treated group,

suggesting that there is a persistence in spatial memory deficits, even after 30 days of withdrawal. Metabolomics data analysis revealed significant increase in histamine metabolites in the 30-day NW group as compared to the saline group. Confirmatory western blot analyses of histamine pathway supported these metabolomic findings. Nicotine-induced global metabolomic changes in the brain may persist after NW and could be responsible for exacerbating ischemic brain damage and cognitive deficits in female rats.

Follow on Funding: Veteran's Affairs, Ami Raval and Helen Bramlett, 10/1/22-9/31/26, Merit Review, Funded: $1.2 million

Collaborations: None at the time of reporting.

Journals: Reddy V, Wurtz M, Patel SH, McCarthy M and Raval AP. Oral contraceptives and stroke: Foes or friends. accepted for publication in journal of Frontiers of Neuroendocrinology

Siegel J, Patel SH, Mankaliyea B and Raval AP. Impact of Electronic Cigarettes Vaping on Cerebral Ischemia: What We Know So Far. Translational Stroke Research 2022. Translational Stroke Research 2022 Apr 18. DOI: 10.1007/s12975-022. PMID: 35435598

Rehni AK, Cho S, Zhang Z, Zhao WZ, Raval AP, Perez-Pinzon MA and Dave KR. Chronic Nicotine Exposure Increases Hematoma Expansion following Collagenase-Induced Intracerebral Hemorrhage in Rats. Biomolecules 2022 Apr 21;12(5):621DOI:10.3390/biom12050621. PMID: 35625548

Reeves MJ, Gall SL, Raval AP. Hello Authors! We Are the Technical Reviewers and Are Here to Help You! Stroke. 2022 Feb;53(2):307-310. DOI:10.1161/STROKEAHA.121.035647. PMID: 34963301

Reddy V, McCarthy M, Raval AP. Xenoestrogens impact brain estrogen receptor signaling during the female lifespan: A precursor to neurological disease? Neurobiol Dis. 2021;163: 105596.DOI: 10.1016/j.nbd.2021.105596. PMID: 34942334

Blaya MO, Raval AP and Bramlett HM. Traumatic brain injury in women across lifespan. Neurobiol Dis. 2022 Jan 4; 164: 105613.DOI: 10.1016/j.nbd.2022.105613.PMID: 34995753

Garcia S, Saldana-Caboverde A, Anwar M, Raval AP, Nissanka N, Pinto M, Moraes CT, and Diaz F. Enhanced glycolysis and GSK3 inactivation promote brain metabolic adaptations following neuronal mitochondrial stress. Hum Mol Genet. 2022 Mar 3;31(5): 692-704.DOI: 10.1093/hmg/ddab282.PMID: 34559217

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 second year of the study, recruitment was completed for Aim 1, the focus group aim, with a total of 44 participants. It was finished short of the target of 65 participants

because saturation of the themes was reached in the iterative analysis of the qualitative data. The three-month follow-up surveys were completed for 40 of the 44 participants in April 2022. Four participants were lost to follow up. With each new focus group, the feedback and data were incorporated in the ongoing analyses. The final results confirmed those reported previously, and the top rated labels are the following and have been revised according to focus group feedback to be used in Aim 2: #5 (Waterpipe Harm Compared to Cigarettes theme; increased risk of cancer), #12 (Health Risks theme; increased risk of oral diseases), #20 (Quitting WP Smoking theme; effect on infants), and #21 (Waterpipe-Specific Harm; increased risk of oral diseases). Parallel to the focus groups, the researchers completed the three "wave" online survey being conducted, with a total of 137 who initiated participation. Fifty-four participants completed the individual ratings, 44 of those also completed the post rating follow up, and 34 were reached and completed the three-month follow up assessment. To assist with conducting an online experimental study to further test the developed labels after their improvement based on results from focus group study, a contract was executed with Kantar, Inc. Two groups (150 each; total 300) of adults (age 21-35 years old) were recruited who are either current waterpipe smokers (Group 1; defined as smoked waterpipe at least once in the past 30 days), or nonsmokers (Group 2; defined as have not used waterpipe in the past year) in a 2x7 between/within subject factorial online experimental study. This online experimental study has also been completed.

Aim 2 is ready to begin in the next grant period at Dr. Maziak's Lab at Florida International University. Dr. Schmidt, design consultant, has conducted quantitative analysis on survey data as well as qualitative analyses of all focus group data to date, including looking at data stratified by gender and other factors. Enrollment of study participants will start in August 2022. The research team has finalized and prepared a new expanded lab space with needed equipment and performed all necessary topography software updates and technical connection checks, and hired a new research nurse to replace the previous research nurse/project/lab manager. Two amendments have been requested for aim 2. The first was to increase participant's incentives to $50 (instead of $36). This amendment was approved by the Department of Health. In the second amendment (still pending), it was requested to remove the plasma nicotine assessment from the battery of measurements done in the lab study of waterpipe health warning labels.

Two accepted abstracts were presented virtually at the 2022 Annual Meeting of the Society for Research on Nicotine and Tobacco (SRNT) as poster presentations. The first focused on analyses from quantitative data collected from the online rating survey of health warning labels. The second abstract focused on qualitative data collected during the focus groups as well as quantitative from the surveys of those focus group participants. A project website has been finalized and published to disseminate knowledge about the harmful effect of waterpipe smoking, publish the developed health warning labels for others to use and test, and to promote the study and its findings. The research team continues to meet regularly with the consultant Mr. Abrams and his colleagues in Golin to create new content for the website as well as to add key events that may be of interest to tobacco control researchers and to present information about this study. The Golin team developed a press release about the overall progress of the study, focus group results, the final warning labels to be tested in the lab, and shared to their newswire, the Florida-specific newswire, and partners at healthcare organizations throughout the state of Florida. Thus far, the press release shared to their newswire has 3,000 thousand views and has matched with 324 sources including Yahoo, MarketWatch, and PR Newswire amongst others. Most of these organizations are broadcast media (67.3%), followed by newspapers (19.4%), and online news sites and other influencers (7.4%). An approximate potential audience of 188,021,574 individuals is expected to be reached.

Follow on Funding: None at the time of reporting.

Collaborations: Researchers continue to meet monthly with the researchers at Florida International University (FIU) according to the executed agreement to finalize and discuss the health warning labels, study procedures, recruitment methods, and the project website. Recruitment for Aim 2 will start in the next month at FIU under the direction of Dr. Wasim Maziak. University of Memphis: Dr. Michael Schmidt, graphic design expert, has completed the analyses of the qualitative data from all focus groups. Researchers are incorporating his proposed image revisions to the most poorly rated health warning labels are working as a group to finalize the text revisions to those labels in the next grant period. Dr. Schmidt's graduate students used the Golin team's work and the Truth Initiative campaign as an example to create new content and/or present existing content in a new way as it relates to smoking. These projects will be another method of dissemination of information about the study and will be included on the website in the next grant period. Truth Initiative: The researchers have continued their collaboration with their consultants at the Truth Initiative, keeping them up to date on the study's progress. During this next grant period researchers will work more closely with them to gather their feedback on the finalized labels and work with them to disseminate the Aim 1 findings.

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 Perez-Pinzon, PhD

Organization: University of Miami

Abstract: The researchers induced a chronic physical exercise (PE) paradigm of three days/week for one month after a model of stroke [middle cerebral artery occlusion (MCAo)] and followed the experiments for another month. Researchers changed the PE paradigm to closely resemble potential clinical scenarios. The research team also observed that five consecutive days of exercise added some stress to post-MCAo animals, an important effect influencing these studies using aged rats. The following groups (n = 10) were used: Group 1: MCAo + control PE (0m/min); Group 2: MCAo + PE (10 m/min for males); Group 3: Sham MCAo + control PE (0m/min); Group 4: Sham MCAo + PE (10 m/min for males). One month later, animals were tested for cued fear conditioning (CFC).

In contrast to previous findings where acute PE was able to improve cognitive outcomes following MCAo, this milder, chronic PE (3 days a week, for 1 month) was not able to produce a significant cognitive improvement. There was no effect on acquisition of fear conditioning (FC), acquisition of extinction, or extinction test. However, PE alone seems to slightly improve acquisition of FC (Day 1) and produce a significant acquisition of extinction (Day 2) in sham MCAO rats.

The researchers are in the process of carrying out immunohistochemical assessments from these animals, and brain samples have been preserved for these assessments. This is a long process of staining and counting and are still counting all nuclei linked to the limbic system in learning and memory (septal nuclei, diagonal band and, anterior and reticular thalamic nuclei).

Neuropathological differences will be compared between the acute PE treatments verses this chronic treatment to define differences in efficacy in both treatments.

Because of the negative cognitive findings using this chronic paradigm, the research team has focused back on the original paradigm of five consecutive days of physical exercise and waited a period of recovery of three months in both male and female rats. These experiments are underway, but currently the labs have moved to a new location on the medical campus. Because of this, a gap of few weeks resulted for this long experiments. The new facilities are completed and are up and running the new set of experiments.
In addition, a pilot study to determine the feasibility for single cell snRNAseq in rat hippocampus has been carried out. Rats were anesthetized and transcardially perfused with ice-cold phosphate-buffered saline (PBS) after the final day of PE. The 4 groups were sham MCAo (+PE or sham PE), and MCAo (+PE or sham PE). Brains were extracted and transferred to dissection medium (Hibernate + DNAse I). Hippocampus was manually dissected, minced and dissociated with Accutase and DNAse I. The suspension filtered through a 35 µm cell strainer. The resulting single-cell suspension was subjected to single-nuclei isolation according to the 10x Genomics Single Cell protocols including myelin removal and sucrose gradient. The researchers are in the process of identifying differentially expressed genes and cell-type specificity of PE response.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

James and Esther King Biomedical Research Program
Appendix L
Fiscal Year 2021-2022 Active Grants
Funded Fiscal Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
9JK01	Florida State University	Gloria Salazar, PhD	$805,409.00	2/28/23	Yes	No	No
9JK02	H. Lee Moffitt Cancer Center and Research Institute	Shelley Tworoger, PhD	$504,838.00	8/31/22	Yes	Yes	No
9JK03	Baptist Health South Florida	John Diaz, MD	$700,000.00	12/31/24	No	No	No
9JK04	University of Central Florida	Alicja Copik, PhD	$805,409.00	3/31/23	No	Yes	No
9JK05	University of Florida	Ramzi Salloum, PhD	$404,909.00	3/31/23	Yes	Yes	No
9JK06	University of Florida	Maria Zajac-Kaye, PhD	$805,409.00	3/31/23	No	No	No
9JK07	University of Miami	Sundaram Ramakrishnan, PhD	$805,393.00	2/28/23	No	Yes	No
9JK08	University of Miami	Kunjan Dave, PhD	$805,409.00	3/31/23	No	Yes	No
9JK09	University of Miami	Nipun Merchant, MD	$805,409.00	3/31/23	No	Yes	No
9JK10	University of South Florida	Rex M. Philpot, PhD	$771,341.00	1/31/23	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. The hypothesis tested in this proposal was 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 research team 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. The progress during this past year was delayed due to the closing of the research labs and offices at Sandels building in January 19, 2022 due to biosafety concerns. The building was reopened for research and teaching in Fall 2022. Dr. Salazar's lab was relocated to a new building in July 2022. After relocation of equipment, the lab became operational at the beginning of September 2022. Due to these delays, the research team only collected data for the experiment testing the effect of blackberry supplementation in atherosclerosis induced by smoking (Aim 3). Data analysis started in September 2022.

Additionally, the effect of cigarette smoke and nicotine in autophagy and the expression of the autophagy adaptor SQSTM1 was uncovered, which was not proposed in the original award. This is rather a new finding derived from this award. Autophagy is a protective process in which the cell eliminates dysfunctional old proteins and organelles to maintain the cell healthier. The process of autophagy is altered during aging and in conditions of disease. The research team

discovered that the defects in autophagy induced by cigarette smoke caused increased oxidative stress and cell toxicity. Additional data will be collected in order to submit the manuscript to a specialized journal for autophagy.

An additional finding was related to vaping and menthol, which was not proposed in the original award. Vaping juice alone increased atherosclerosis in mice, which was further elevated by nicotine in vaping juice. Menthol reduced atherosclerosis in a sex-dependent manner. Menthol is more effective in reducing atherosclerotic plaque in male than in female mice. Further, nicotine and menthol remodeled the intestinal microbiome. The microbiome refers to the bacteria living in the intestine. These bacteria have a profound effect in human health. Health-promoting bacteria protects against disease, while disease promoting bacteria causes inflammation, oxidative stress and negatively alter lipid and carbohydrate metabolism. In summary, this award opened new avenues of research that will be explored in future research. Several grant proposals were submitted to explore these new findings.

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 Tworoger, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: Ovarian cancer is the deadliest gynecological cancer in Florida and is responsible for nearly 1,000 deaths annually statewide. Increasing evidence suggests 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 reporting period, research staff completed analyses related to the primary aims of the award, including analyses of early life physical activity and adversity in relation to risk of ovarian cancer. With regard to the secondary aims, analyses of early life smoking exposure in relation to ovarian tumor immune profile were completed.

A manuscript describing findings of early life physical activity and ovarian cancer risk was published in the December 2021 issue of the International Journal of Cancer (IJC). For analyses of early life abuse, the study team combined results from the Nurses' Health Studies (NHS, NHSII), Black Women's Health Study, and Sister Study. Analyses of early life socioeconomic status pooled data from NHS and NHSII. Overall, the research staff did not observe associations of ovarian cancer risk with early life sexual or physical abuse or socioeconomic status, however, researchers could not rule out a modest increased risk for women with a history of sexual abuse that occurred four or more times. Results of analyses of smoking and ovarian tumor immune profile indicated early life cigarette smoke exposure is associated with increased risk of developing ovarian tumors with low abundance of total T cells and low abundance of recently activated cytotoxic T cells. Manuscripts describing these results are in preparation.

There are several take-home messages from the research completed during the reporting period. First, given prior evidence that depression and post-traumatic stress disorder increase risk of ovarian cancer, the study team's results suggest the stress response has greater impact on ovarian tumor development than stressors alone. For Floridians, this suggests there is an opportunity for intervention to reduce distress in girls and women who have experienced abuse as well as to prevent abuse from ever occurring in the first place. In addition, results consistent with a diminished immune response to developing ovarian tumors among women who were exposed to smoking as children supports continued work to create effective smoking cessation programs targeted to parents.

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. There are currently no University of Florida students performing research under this research project. Harvard T.H. Chan School of Public Health: Jennifer Mongiovi, PhD, a postdoctoral research fellow in the Department of Epidemiology at the Harvard T.H. Chan School of Public Health, is performing research under this research project.

Journals: Wang, T., Jake-Schoffman, D.E., Townsend, M.K., Vinci, C., Willett, W.C., Tworoger, S.S., Early life physical activity and risk of ovarian cancer in adulthood, Int. J. Cancer, 2021, 149: 2045-2051. DOI: 10.1002/ijc.33760 PMCID: PMC8542620.

Patents: None at the time of reporting.

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

Principal Investigator: John Diaz, MD

Organization: Baptist Health South Florida

Abstract: The James and Esther King Grant has supported this clinical trial evaluating Immunotherapy in combination with PARP (a cellular enzyme blocking substance) inhibition in advanced cervical cancer patients functionally competent or deficient for the Fanconi Anemia repair pathway. The clinical trial provides women suffering from recurrent cervical cancer access to novel therapies combining pembrolizumab, immunotherapy, with olaparib, PARP inhibitors. Additionally previous data demonstrated a possible new biomarker in the Fanconi Anemia repair pathway that may predict a response to immunotherapy. The researchers continue to enroll patients onto this clinical trial; however, enrollment has been slowed due to challenges from the COVID pandemic. These challenges include: a decrease in patients visits to physicains to seek medical care and research staffing shortages. In an effort to increase enrollment, partnerships with other academic institutions in the state of Florida are being explored. The researchers' collaboration with Dr. Wenrui’s laboratory at Florida International University has been interrupted due to funding for Dr. Wenrui’s laboratory. The researchers continue to collect samples for their translational research component. Dr. Wenrui is currently establishing his laboratory at another institution within the state. This research continues to receive drug support from Merck and Astrazeneca. The research team continues to partner with local grassroots organizations such as The Promise Fund to recruit patients for the clinical trial. Cervical cancer disproportionately impacts minority women and those of lower socioeconomic

status. This population has traditionally been less represented in clinical trials, as such continued effort has been made to reach this population in South Florida. During this time, ten patients were identified as eligible for the trial and successfully enrolled six patients. At this time, one patient is actively receiving treatment and additional patients are in the screening process. The researchers are actively establishing the infrastructure at their institution to lead multicenter clinical trials. Simultaneously, the research team is identifying potential collaborators in Florida who would be interesting in opening the trial at their institution. This will allow an increased pool of patients within the state of Florida.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Alicja 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 the laboratory and reprogrammed to be highly activated through exposure to cellular plasma membrane particles (PM21) or exosomes (EX21) derived from IL21 expressing feeder cells. These cells can be further edited to improve their function and thus potential anti-tumor efficacy which is part of the current work. During this current reporting period work was done that demonstrated that cryopreserved NK cells are as good as fresh NK cells. Cryopreservation is critical for developing NK cells as a cellular therapeutic. The results of this work was published in Frontiers in Immunology. The gene editing methodology has been optimized and used to knock out a receptor called TIGIT that is present on PM21-NK cells in high abundance and was hypothesized to block NK cell function. Work performed as part of this grant demonstrated that this receptor negatively impacts PM21-NK cell function and genetic modification of NK cells to remove TIGIT can greatly enhance tumor killing. These modified cells have potential to be therapeutically more effective when used alone or in combination with newly developed therapeutics targeting this molecule. The patent was licensed to Kiadis Pharma, a Sanofi company for clinical development. As part of this funding PM21-NK cells were also modified to remove another protein called PD-L1. These modified NK cells kill tumors well and are resistant to self-destruction when combined with immunotherapeutic antibody called Avelumab. Collectively, the results of this work were presented at conferences, patented and will be published. These modified NK cells will likely be undergoing clinical development and if approved will benefit Floridians.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Oyer, J.L., Croom-Perez, T.J., Dieffenthaller, T.A., Robles-Carillo, L.D., Gitto, S.B., Altomare, D.A., Copik, A.J. Cryopreserved PM21-particle-expanded Natural Killer cells maintain cytotoxicity and effector functions in vitro and in vivo. Front Immunol. 2022; 13:861681. DOI:10.3389/fimmu.2022.861681
https://www.frontiersin.org/articles/10.3389/fimmu.2022.861681/full

Croom-Perez T.J., Robles-Carillo, L.D., Dieffenthaller, T.A., Oyer, J.L., Hasan, Md F and Copik A.J Kinetic, "Imaging Based Assay to Measure NK Cell Cytotoxicity Against Adherent Cells" Methods in Cell Biology Book series- accepted

Patents: Copik AJ, Hasan MF, Croom-Perez TJ “Engineered NK Cells and Uses Thereof”- provisional patent application was filed on 09/29/2021

Copik AJ, Croom-Perez TJ, Oyer JL, Dieffenthaller TA, Hasan MF, Robles-Carillo LD “PD-L1 KO NK Cells For Use with Pd-L1 Targeting Treatments” - provisional patent application was filed on 04/08/2022

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

Principal Investigator: Ramzi Salloum, PhD

Organization: University of Florida

Abstract: The purpose of this grant is to evaluate the feasibility of reducing secondhand tobacco smoke exposure in children within the pediatric care setting. This project takes place at the pediatrics clinics from the University of Florida Health System: Tower Square, Children's Medical Services (CMS), Magnolia Parke, Tioga, Main Street, East Side, and pediatric specialty clinics. During the past funding period, the research team continued the recruitment process at the participating clinics. During the recruitment process, the research team worked in collaboration with social workers at the clinics to identify potentially eligible participants. The research team had collected a total of 1,778 completed questionnaires, and have successfully enrolled a total of 60 patients with tobacco exposure that were eligible for a patient exit interview (PEI). The PEIs were administered electronically via Research Electronic Data Capture (REDCap) after each visit, and were used to assess provider counseling at intervention and usual care clinics, and as a measure of fidelity in intervention clinics. A total of 39 patients have completed the three-month follow up questionnaire. The research team is continuing to administer the three-month follow up questionnaires and are no longer recruiting new patients. The research team also began developing a protocol manuscript and have a near-final version of the paper. In summary, significant progress was made during the past funding period in regards to identifying and recruiting patients, and in administering follow up questionnaires in order to fulfill the aims of determining the feasibility and efficacy of a tobacco control intervention, and identifying the predictors of reach among patients and parents who use tobacco.

Follow on Funding: Aetna Foundation, Ramzi Salloum, PhD, 3/1/20-10/31/22,Aetna Foundation Research Grant, Funded: $225,000

Collaborations: The study is to be conducted entirely at the University of Florida and there are currently no relevant collaborations to report with other postsecondary educational institutions. This study is led by Dr. Ramzi G. Salloum from the College of Medicine at UF.

Journals: Copik AJ, Hasan MF, Croom-Perez TJ "Engineered NK Cells and Uses Thereof"- provisional patent application was filed on 09/29/2021

Copik AJ, Croom-Perez TJ, Oyer JL, Dieffenthaller TA, Hasan MF, Robles-Carillo LD "PD-L1 KO NK Cells For Use with Pd-L1 Targeting Treatments" - provisional patent application was filed on 04/08/2022

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 finalize the antitumoral and survival studies in the research team's newly established pancreatic ductal adenocarcinoma (PDAC) genetically engineered mouse models (GEMM). The team tested the effect of the maximum possible dose of novel drug compounds to inhibit thymidylate synthase (TS) for treatment of pancreatic cancer. The principle investigator's laboratory demonstrated that pancreatic overexpression of TS (essential enzyme for DNA synthesis and repair aberrantly overexpressed in a range of human cancers) promoted aggressive PDAC development and markedly reduced survival of genetically engineered KRAS-mutant (an error in a protein) mice. Thus, the goal of this proposal is to develop new treatments for pancreatic cancer using unique TS inhibitors identified in the laboratory. Since the researchers' preclinical data show that TS inhibitors synergistically enhance RAS/PI3K/AKT/mTOR inhibition in vitro, it is proposed in this project to test new TS inhibitors alone or in combination with mTOR inhibitors using their novel hTS/Kras and hTS/Kras.Pten PDAC GEMM models and patient derived xenografts (PDX).

In the past year, two survival studies in hTS/Kras and hTS/Kras.Pten GEMM have been finalized to determine the antitumoral effect of compound P, Everolimus and the combination of both. Compound P (also known as Mefloquine) and Everolimus (mTOR inhibitor) are both FDA approved drugs and used for different indications. Compound P for the treatment and prevention of malaria; and Everolimus for advanced cancers and non-cancerous tumors. The researchers established that 200 MPK compound P may prolong mice survival and that a dose of 5 MPK Everolimus is too potent to increase compound P efficacy in GEMMs. Therefore, new survival studies in hTS/Kras and hTS/Kras.Pten GEMM were initiated with lower doses of both drugs to determine the antitumoral effect of 100 MPK compound P and 2.5 MPK Everolimus with the goal of further increase the potency of compound P. It was also determined that 2 other mTOR inhibitors, Temsirolimus (a rapalog inhibitor of mTOR) and TAK-228 (a highly selective mTORC1/TORC2 inhibitor) have a potent cytotoxic effect alone that induces synergy in vitro when combined with compound P. In addition, the researchers established the maximum tolerated dose for both mTOR inhibitors alone and identified the dose that will be used for studies in vivo.

To determine if this new drug combination shows differential responsiveness between smokers and non-smokers, the researchers expanded 7 human samples (collected from pancreatic

biopsies from smokers and non-smokers) into PDX and 2 were tested for sensitivity with 100 MPK compound P. The effect of compound P alone resulted in a potent antitumoral effect as compared to untreated controls. Testing the efficacy of 100 MPK compound P and 2.5 MPK Everolimus in smokers and non-smokers will continue to determine if there is a differential response to treatment among both population groups.

This work will lay the groundwork for a personalized investigator-initiated clinical trial at the University of Florida that will reduce PDAC mortality in the Floridian population. Better understanding of this targeted drug combination will enable to treat PDAC patients, improve quality of life and clinical outcomes.

Follow on Funding: None at the time of reporting.

Collaborations: In the last year, University of Florida undergraduate student receiving training and performing research under this project were Allison Cama, Liz Sanders, Alejandra Iglesias and Madeline Gubbini.

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. Previously, the researchers investigated the impact of a cigarette smoke-induced hypoxia on pancreatic cancer cells. Tumor hypoxia leads to metabolic adaptation and resistance to chemotherapy. Therefore, understanding the effect of hypoxia-induced changes in pancreatic cancer under the influence of cigarette smoke (constituents) is highly warranted. In earlier studies, the researchers have characterized the role of micro-RNAs (miR) regulating fine-tuning metabolic pathways of tumor cells. One of the important miRs upregulated during hypoxia is miR-210. The researchers also investigated the impact of miR-210 in pancreatic cancer progression in an orthotopic model. Deletion of miR-210 in pancreatic cancer cells increased in vitro proliferation and tumor growth in vivo. Increased tumor growth was seen in ahtymice mice as well indicating that an intact immune system is not contributing to the observed larger tumors seen in miR-210 deleted KPC cells. In the future, the researchers will investigate the role of microbial metabolites in miR-210 KO models and evaluate the metabolite contribution to pancreatic cancer growth and metastasis.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Sundaram Ramakrishnan. HIF-2 in cancer-associated fibroblast polarizes macrophages and creates an immunosuppressive tumor microenvironment in pancreatic cancer. Gastroenterology, 2022: ePublication March 28. DOI: https//doi.org/10.1053/j.grastro.2022.03.035

Charles Jacob HK, Charles Richard JL, Signorelli R, Kashuv T, Lavania S, Vaish U, Boopathy R, Middleton A, Boone MM, Sundaram Ramakrishnan, Dudeja V, Saluja AK. Charles Jacob HK, et al. Modulation of Early Neutrophil Granulation: The Circulating Tumor Cell-Extravesicular Connection in Pancreatic Ductal Adenocarcinoma Cancers (Basel). 2021 May 31;13(11): 2727.DOI:10.3390/cancers13112727. Cancers (Basel). 2021. PMID: 34072942

Patents: None at the time of reporting.

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

Principal Investigator: Kunjan 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 a large number of patients between three and 24 hours after onset. Hematoma volume in sICH patients correlates with the 30-day mortality rate. Currently, there is no proven therapy to prevent hematoma expansion in sICH patients, and thus clinicians are unable to offer more than supportive care. Several epidemiological studies demonstrated the deleterious effects of smoking/tobacco use in sICH patients. These effects include increased risk of sICH, larger hematoma expansion, and poor post-sICH outcomes. Despite several clinical studies indicating the deleterious effects of smoking/tobacco use in sICH patients, the field is lacking confirmatory systematic preclinical studies evaluating the effects of smoking on outcomes following sICH. The main goal of the proposal is to achieve the goals of the James and Esther King Biomedical Research Program by improving the health of Floridians. In this project, the research 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. The team 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, 1) confirmed that chronic nicotine treatment in aged male rats resulted in impaired blood-brain barrier integrity (functional and protein levels); 2) red blood cell microparticle treatment was able to lower hematoma growth and improve neurological deficits following spontaneous hemorrhagic stroke in nicotine exposed young female rats when treatment was started up to 4.5 h post-hemorrhage; 3) treatment with a tumor necrosis factor(TNF)-alpha inhibitor reduced chronic nicotine exposure-induced deficits in blood brain barrier integrity (functional and protein levels) in young male and female rats; 4) treatment with a TNF-alpha inhibitor reduced hematoma growth and improved neuroligical deficits in chronic nicotine-treated young female rats following collagenase-induced sICH; 5) confirmed that chronic nicotine treatment in aged female rats resulted in impaired blood brain-barrier integrity (functional and protein levels); and 6) red blood cell microparticle treatment limit hematoma expansion insICH by enhancing secondary hemostasis through both the intrinsic and extrinsic pathways.

Follow on Funding: None at the time of reporting.

Collaborations: Dr. Ashish K. Rehni: Post-doctoral Associate, Ms. Sunjoo Cho: Research Associate, Ms. Snigdha Reddy Sama: Undergraduate student, and Ms. Priyanka Khushal: Undergraduate student.

Journals: 1) Ashish K. Rehni, Sunjoo Cho, Zhexuan Zhang, Weizhao Zhao, Ami P. Raval, Miguel A. Perez-Pinzon, Kunjan R. Dave, Chronic Nicotine Exposure Increases Hematoma Expansion Following Collagenase-Induced Intracerebral Hemorrhage in Rats. Biomolecules, 2022 Apr 21;12(5):621.

2) A. K. Rehni, S. Cho, H. Navarro Quero, V. Shukla, Z. Zhang, Y. S. Ahn, C. Dong, W. Zhao, M. A. Perez-Pinzon, S. Koch, W. Jy, K. R. Dave. Red blood cell microparticles limit hematoma growth in intracerebral hemorrhage. Accepted for publication in journal Stroke.

Patents: None at the time of reporting.

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

Principal Investigator: Nipun Merchant, MD

Organization: University of Miami

Abstract: Pancreatic cancer remains a major health burden associated with an abysmal 5-year survival rate (<11%) and poses a major therapeutic challenge. There has been a steady rise in the number of pancreatic cancer patients particularly in South Florida and these numbers are projected to increase in the coming decade. Out of 53,000 cases of pancreatic cancer in US alone, about 11% are from Florida according to the Surveillance, Epidemiology, and End Results (SEER) database. While most of the other cancers like breast cancer and lung cancers have a projected decline in incidence in the next 10 years, pancreatic cancer incidence has increased, threatening to become the second most common cancer in United States. Epidemiological studies reveal that ethnicity as well as lifestyle choices like tobacco smoking contributes to the risk factor in a disease. According to CDC, about 15-18% of the population of Florida are tobacco users. This significantly raises the risk for pancreatic cancer in this state. The pancreatic tumor microenvironment is characterized by the presence of a robust, fibroinflammatory stroma. During tumor progression, this microenvironment develops anatomically distinct “niches”. This leads to enrichment of a small population of cells that have a distinct survival advantage over the bulk of the tumor cells, contributing to the aggressive biology of the disease. The role of the stromal cells in the pancreatic tumor microenvironment has gained importance in the last decade. Yet, how the stroma influences the properties of the tumor has remained relatively unknown. The research team's work has shown that in pancreatic tumors, the developing stroma increases tumor initiation frequency in pancreatic cancer cells in vivo by enriching for CD133 + aggressive “stem-like” cells. Furthermore, the stromal fibroblasts secrete IL6 as the major cytokine which increases glycolytic flux in the pancreatic tumor cells and increases lactate efflux in the microenvironment via activation of the STAT signaling pathway. The researchers also show that the secreted lactate favors activation of M2 macrophages in the tumor microenvironment, which excludes CD8 + T cells in the tumor. Additionally, researchers have shown that treatment with anti-IL6 antibody results in tumor regression as well as decreased CD133 + population within the pancreatic tumor. Subsequent inhibition of lactate efflux cause M2 to M1 polarization of macrophages in pancreatic tumor and thereby inducing anti-tumor immunity and making pancreatic tumors to more responsive to anti-PD1 immunotherapy. Furthermore, the researchers have shown that stroma mediated

metabolic programming cause significant secretion of lactate which makes tumor microenvironment slightly acidic. Their metabolic mass spectrometric analysis showed that promiscuous activity of LDH under acidic environment produces L-2HG in pancreatic tumor cells as well as stromal cell. This abnormal accumulation of L-2HG led to deregulated histone demethylation to suppress pro-differentiation gene and activate stemness genes expression, thereby regulating a critical balance between stemness and differentiation in pancreatic tumors. Overall, their work has shown that stromal IL6 driven metabolic reprogramming plays a crucial role in the development of an immune-evasive microenvironment and in vivo targeting of these metabolic pathways make pancreatic tumors amenable to checkpoint inhibitor therapy which will improve overall survival among pancreatic cancer patients.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Kesh K, Mendez R, Mateo-Victoriano B, Garrido VT, Durden B, Gupta VK, Oliveras Reyes A, Merchant N, Datta J, Banerjee S, Banerjee S. Obesity enriches for tumor protective microbial metabolites and treatment refractory cells to confer therapy resistance in PDAC. Gut Microbes. 2022 Jan-Dec;14(1):2096328. DOI: 10.1080/19490976.2022.2096328.

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: The researchers have learned that MMTV-PyVT breast cancer tumor bearing mice do not perform as well during assessment of baseline cognitive function as non-tumor bearing matched controls, which parallels reports in the human literature that cancer patients often exhibit some cognitive impairments prior to cancer treatment with chemotherapy. The researcher team has established that the doses of 6.7mg/kg (≈20mg/m2) doxorubicin (DOX) and 66.7mg/kg (≈200mg/m2) cyclophosphamide (CYP) are effective at attenuating tumor number and growth in the MMTV-PyVT breast cancer mouse model for the duration of their behavioral assessment paradigm. Further, it has been determined that repeated administration of these doses leads to a deficit in cognitive function that persists after the termination of treatment. Additionally in non-tumor bearing mice, it has been determined that both acute and repeated administration of DOX and CYP results in increases in circulating concentrations of macrophage-inflammatory protein 2 [MIP-2 (CXCL2)] that persist after the end of exposure, while tumor bearing mice exhibit higher circulating concentrations of MIP-2 (CXCL2) than non-tumor bearing mice in general. This is important because MIP-2 (CXCL2) has been: 1) demonstrated to impair cognitive function; 2) shown to cause white matter damage, often observed in studies of chemo brain; and 3) associated with long term cognitive deficits. These findings indicate that circulating concentrations of monocyte chemoattractant protein-1 [MCP1 (CCL2)], which promotes lung metastasis in breast cancer, is elevated in tumor bearing mice and attenuated by chemotherapy and that following chemotherapy, circulating concentrations of this protein increase relative to tumor bearing mice that have not received chemotherapy. Elevated concentrations of MCP1 (CCL2) are associated with both a greater severity of, and faster cognitive decline in, Alzheimer's disease. Therefore, this post chemotherapy spike in MCP1 (CCL2) may be a contributor to the persistence of cognitive impairment associated with

chemotherapy treatment. Lastly, when either non-tumor or tumor bearing mice exposed to chemotherapy are administered xanolamine, at 1.8 mg/kg, or VU-357017 at 1.0 mg/kg, the mice do not exhibit a decline in cognitive function during or following chemotherapy and perform better than saline treated 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.

James and Esther King Biomedical Research Program
Appendix M
Fiscal Year 2021-2022 Active Grants
Funded Fiscal Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
8JK03	H. Lee Moffitt Cancer Center and Research Institute	Nagi Kumar, PhD	$700,000.00	3/31/23	No	No	No
8JK04	University of Florida	Frederic J. Kaye, MD	$805,409.00	3/31/23	No	No	No
8JK06	University of Florida	Sergei G. Tevosian, PhD	$816,514.00	9/30/21	Yes	No	No
8JK09	University of South Florida	Tomar Ghansah, PhD	$816,514.00	3/31/21	Yes	Yes	Yes

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

Principal Investigator: Nagi Kumar, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

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. The researchers 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 proresolving properties towards resolution of cigarette smoke-induced lung inflammation in former smokers. The researchers 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 are administered independently. Based on this evidence, the researchers hypothesize that a standardized formulation of CUR + ω-3 FA will target molecular pathways that are critical for lung cancers development, leading to a reduction in the overall size and density of nodules, in former smokers. It is further hypothesized that this will be mediated by reducing inflammation and through pro-resolving effects in the nodules. Hypothesis will be tested by using an experimental design and rigorously evaluating the safety, efficacy and validate the potential mechanism of a combination of ω-3 FA + CUR or placebo administered for 6 months in former smokers, age ≥55 years, with lung nodules detected during LDCT screening program. Results of the proposed trial may have immediate and significant benefit to former smokers and other high-risk populations towards lung cancer prevention. In spite of the reduced funding, the goal is to obtain the safety and effectiveness of the combination of combination of ω-3 FA + CUR or placebo in 100 men and women who are diagnosed with the lung nodules. Although the researchers have faced several challenges in recruiting subjects due to the pandemic and subjects who qualify for the trial are hesitant to enroll in study due to the need to travel, the team has continued to expand the eligibility criteria and have revised the protocol to minimize the risk to potential subjects to improve recruitment in this trial and accomplish the work proposed.

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: Frederic J. Kaye, MD

Organization: University of Florida

Abstract: Small cell lung cancer (SCLC) is the tumor subtype that is most tightly associated with tobacco exposure and represents a clinical and genetic tumor that is unique from all other types of lung cancer. Most importantly, SCLC represents the most lethal type of lung cancer with no meaningful improvements in treatment regimens over the past 40 years and negligible survival for patients who present with advanced disease. For example, while advances in cancer gene mutational testing in other non-SCLC tumor types have identified new targeted treatment opportunities that improve patient survival, there are currently no “actionable” cancer gene mutations for SCLC. In addition, new immunotherapy strategies have been disappointing, and the FDA recently withdrew recommendations for the use of the immunotherapy agent, pembrolizumab (Keytruda), in advanced SCLC. The researchers' laboratory has been focused on testing a new oncolytic viral therapy to investigate if this strategy might induce an enhanced host immune cell response to improve tumor response, especially if delivered concurrent with chemotherapy or immunotherapy regimens. The team recently published preclinical data supporting the delivery of a unique modified myxoma viral agent (MYXV) to stimulate the immune system, to induce tumor specific cell death, and to improve survival in animal models of SCLC. This data was published in one of the top medical journals with high citation impact (impact factor 19.456; Oncolytic virotherapy for small-cell lung cancer induces immune infiltration and prolongs survival. J Clin Invest 2019; 129(6): 2167-2595). The research team is now working to translate this promising pre-clinical data into a novel investigator-initiated phase 1 clinical trial testing the safety and efficacy of MYXV delivered directly into lung tumor nodules of patients with advanced SCLC. The primary goals over the past 12 months have been to optimize and complete the Good Laboratory Practice (GLP) clinical MYXV product to complete the regulatory requirements for an Investigational New Drug (IND) submission. In the past year, the researchers have worked with the University of Florida Powell Gene Therapy Center to finalize this process. The research team has also worked closely with the FDA to clarify the requirements for animal safety and biodistribution testing. The FDA did not agree with their plan for performing safety testing with standard normal mice and required that animal safety testing be performed using a larger tumor-bearing animal that would allow the research team to closely replicate the delivery strategy of the proposed phase 1 trial. Over the past year, the researchers have worked with the University of Florida College of Veterinary Medicine to perform this testing. This clinical trial proposes a ‘first-in-human’ clinical trial with intratumoral MYXV delivery that represents a multi-Departmental collaboration of clinical and basic science research expertise at the University of Florida College of Medicine. In addition, the researchers have collaborated with investigators at the Moffitt Cancer Center on the pre-clinical testing of MYXV and will continue to pursue collaborations within the State of Florida to test this novel approach to improve the outcome for 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 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.

3. **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: This study was undertaken to explore poorly understood changes in the maternal gut microbiome in response to nicotine exposure during pregnancy. Using a well-established rodent model of prenatal nicotine exposure, researchers hypothesized that prenatal nicotine exposure (PNE) induces changes in the maternal gut-microbiome, which in turn alters fetal exposure to circulating SCFAs and leptin during development and leads to adverse changes in the adult. The key findings obtained during the funding period demonstrate an effect of the nicotine exposure during pregnancy and in the offspring, both during the fetal development and in the adult.

Follow on Funding: None at the time of reporting.

Collaborations: Research staff are currently using unrestricted funds available to continue work on the epigenetics part of the project with Dr. Smagulova. Dr. Smagulova worked as a postdoctoral fellow in Dr. Tevosian's laboratory at Dartmouth in 2005-2007. Dr. Smagulova is now running a lab in France and is the top-notch expert on epigenetic analysis with articles in Nature (e.g., Genetic recombination is directed away from functional genomic elements in mice. Brick K, Smagulova F, Khil P, Camerini-Otero RD, Petukhova GV. *Nature.* 2012 and Genome-wide analysis reveals novel molecular features of mouse recombination hotspots. Smagulova F, Gregoretti IV, Brick K, Khil P, Camerini-Otero RD, Petukhova GV. *Nature*. 2011). This work will be aimed at revealing the mechanistic basis for the physiological consequences of nicotine exposure.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

4. **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: The research team was officially awarded the FLDOH JEK Grant in April 2018. From April 2018 to December 2020, respectively the research team generated all of the data in these years along with full time, part-time staff members and master and undergraduate students. In fact, the team was able to garner the necessary data (i.e. pancreatic cancer [PC] orthotopic mice were treated with and without API and then monitored with Vevo Ultrasound Image system) in order to complete the 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 SHIP KO and SHIP WT mice to improve to reproducibility of the results. The team revised the manuscript 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 research manuscript and submitted to Cancers in August 2020.

Follow on Funding: State of Florida, 4/17/18-9/31/21, $816,514

Collaborations: Jose Trevine, M.D., Margaret Hibbs, Ph.D., Bradford McGwire, MD, Ph.D., Clayton Yates, Ph.D., Cyclica Biotechnology Drug Compant.

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

Patents: Apigenin Increases SHIP-1 Expression, Promotes Tumoricidal Macrophages and Anti-Tumor Immune Responded in Murine Pancreatic Cancer. ID#: 20B161 Approved Date: 09/10/2020, University of South Florida

James and Esther King Biomedical Research
Appendix N
Fiscal Year 2021-2022 Closed Grants
Funded Fiscal Year 2020-2021

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
21K01	University of Miami	Yanbin Zhang, PhD	$100,000.00	11/30/21	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. Therefore, understanding the molecular mechanisms of genome instability in cancer cells is imperative for the development of novel treatment strategies. Fanconi Anemia is a hereditary disorder caused by mutations in at least 22 genes and clinically characterized by bone marrow failure and predisposition to cancer. This proposal focuses on FANCA, a gene that is mutated in ~64% of the entire FA patient population. Based on the preliminary studies, we hypothesize that FANCA promotes genome instability, regulates cell cycle progression, and serves as a vulnerability for cancer intervention. For this reporting period, we have carried out experiments to delineate the role of FANCA in genome instability and cancer development. The resulting data strongly support our hypothesis of how FANCA contributes to genome instability, cell cycle progression, and tumor growth. This project employs a combination of biochemical, cell biological, genetic, and in vivo approaches to study how genome instability leads to cancer, which should ultimately translate into better and more targeted therapies at the bedside for Floridians. Completion of this proposal will define a novel role for FANCA in cancer development, establish the significance of FANCA as a unique rationale-driven therapeutic target for breast cancer treatment, and lead to a transformative approach to treat high FANCA-expression 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.

James and Esther King Biomedical Research
Appendix O
Fiscal Year 2021-2022 Closed Grants
Funded Fiscal Year 2016-2017

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
7JK02	H. Lee Moffitt Cancer Center and Research Institute	Christine H. Chung, MD	$1,896,200.00	2/28/22	Yes	Yes	No
7JK04	H. Lee Moffitt Cancer Center and Research Institute	Jhanelle E. Gray, MD	$1,895,355.00	2/28/22	Yes	Yes	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 and Research Institute

Abstract: The proposed phase I/II clinical trial of cetuximab and nivolumab was completed, and the results were published in Clinical Cancer Research, in June 2022 with a title, “Phase II multi-institutional clinical trial result of concurrent cetuximab and nivolumab in recurrent and/or metastatic (R/M) head and neck squamous cell carcinoma (HNSCC).” Briefly, the researchers determined the efficacy of cetuximab and nivolumab and expression of p16 and programed cell death ligand 1 (PD-L1) in archived tumors. Ninety-five patients were enrolled, and 88 patients were evaluable for overall survival (OS) with a median follow-up of 15.9 months. Median OS in the 45 patients who had prior therapy for R/M HNSCC was 11.4 months, with a 1-year OS 50% (90% CI, 0.43-0.57). Median OS in the 43 patients who had no prior therapy was 20.2 months, with a 1-year OS 66% (90% CI, 0.59-0.71). In the combined cohorts, the p16-negative immunostaining was associated with higher response rate (RR, p=0.02) but did not impact survival while higher PD-L1 combined positive score was associated with higher RR (p=0.03) and longer OS (log-rank p=0.04). The combination of cetuximab and nivolumab was determined to be safe and effective in patients with both previously treated and untreated R/M HNSCC. In addition, to determine the predictive biomarker of clinical benefit, T cell receptors (TCR) were sequenced from 41 patients from the clinical trial using peripheral blood mononuclear cells (PBMC) collected from at least three time points. The major finding is that the patients with more polyclonal TCRB at screening tend to have better response to the combination therapy. An overall trend toward worse patient survival was also observed in the oligoclonal group. The research also found patients with a higher total TCR rearrangements have better survival outcomes, and the never smokers have more diverse TCR repertoire in PBMC compared to current or former smokers. The manuscript with a title, “T cell repertoire in peripheral blood as a potential biomarker for predicting response to concurrent cetuximab and nivolumab in HNSCC,” was published in Journal of ImmunoTherapeutics and Cancer in June 2022. Data regarding patient-reported outcomes (PROs) from the trial with combination of cetuximab and nivolumab as well as immune checkpoint inhibitor (ICI) monotherapy were also obtained from 86 patients and submitted for a publication in Head & Neck which is currently under review. The toxicity index was higher in patients receiving combination therapies at three months and at 18-20 weeks after initiation of treatment compared to those receiving ICI monotherapy; toxicity index scores increased for both groups over time. In both monotherapy and combination therapy groups, overall QOL, emotional well-being, and functional well-being improved from baseline to 12 weeks, with stable or declining QOL thereafter. At three and six month follow-up, there were no significant differences in overall QOL between the monotherapy and combination groups.

Among patients treated with combination therapy, patient-reported moderate to severe toxicities were far more common than Grade three or above clinician-reported toxicities. These findings will improve the health outcome of Floridians with R/M HNSCC.

Follow on Funding: NIDCR, Aik Choon Tan and Christine Chung, 9/2021-8/31/2024, $1,875,380

Collaborations: None at the time of reporting.

Journals: Wang X, Muzaffar J, Kirtane K, Song F, Johnson M, Schell MJ, Li J, Yoder SJ, Conejo-Garcia J, Guevara JA, Bonomi M, Bhateja P, Rocco JW, Steuer CE, Saba NF, Chung CH. T cell repertoire in peripheral blood as a potential biomarker for predicting response to concurrent cetuximab and nivolumab in head and neck squamous cell carcinoma. J Immunotherapy Cancer. 2022; Jun; 10(6): e004512. PMCID: PMC9185557.

Chung CH, Li J, Steuer CE, Bhateja P, Johnson M, Masannat J, Poole MI, Song F, Hernandez-Prera JC, Molina H, Wenig BM, Kumar S, Kuperwasser C, Stephens PJ, Farinhas JM, Shin DM, Kish JA, Muzaffar J, Kirtane K, Rocco JW, Schell MJ, Saba NF, Bonomi M. Phase II multi-institutional clinical trial result of concurrent cetuximab and nivolumab in recurrent and/or metastatic head and neck squamous cell carcinoma. Clin Cancer Res. 2022; Jun. 28(11):2329-2338. PMCID: PMC9167762.

Glazar DJ, Johnson M, Farinhas J, Steuer CE, Saba NF, Bonomi M, Schell MJ, Chung CH*, Enderling H.* Early response dynamics predict early treatment failure in patients with recurrent and/or metastatic head and neck squamous cell carcinoma treated with cetuximab and nivolumab. Oral Oncol. 2022; Apr. 127:105787. PMID: 35248922.

Oswald LB, Brownstein NC, Whiting J, Hoogland AI, Saravia S, Kirtane K, Chung CH, Vinci C, Gonzalez BD, Johnstone PAS, Jim HSL. Smoking is related to worse cancer-related symptom burden. The Oncologist. 2022; Mar. 27(2): e176-e184. PMID: 35641215.

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; Oct. 37(20):3681-3683. PMID: 33901274.

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 and Research Institute

Abstract: In collaboration with the thoracic clinical trials office, a list of patients enrolled for MCC 19406 and evaluable was generated. Through an SQL based query on LabVantage/Bio-banking database, Pre and On-treatment biospecimens including snap frozen, Formalin fixed Paraffin embedded (FFPE) were identified for each of these patients from the list. For germline DNA, whole blood aliquots and Mononuclear cells (MNC) for PBMC studies were also identified. The identified tissues were requested from Tissue Core's Biorepository and were followed up for

further downstream applications. A master list of patients to sample linkage involving PHI was maintained for the sole use of the principal investigator. An inventory list of residual biospecimen collected under MCC 19406 and stored in Tissue Core bank was also maintained.

Follow on Funding: American Society of Clinical Oncology, 7/1/2022-6/30/2023, $50,000

Collaborations: None at the time of reporting.

Journals: Sonam Puri, Sandrine Niyongere, Monica S. Chatwal, Theresa A. Boyle, Dung-Tsa Chen, David Noyes, Scott J. Antonia, Jhanelle E. Gray: Phase I/II study of nivolumab and ipilimumab combined with nintedanib in advanced NSCLC

Patents: None at the time of reporting.

James and Esther King Biomedical Research
Appendix P
Fiscal Year 2021-2022 Closed Grants
Funded Fiscal Year 2015-2016

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Publications	Patents
6JK02	H. Lee Moffitt Cancer Center and Research Institute	Vani N. Simmons, PhD	$1,186,164.00	6/30/21	No	Yes	No

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

Principal Investigator: Vani N. Simmons

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: During the current reporting period researchers were able to complete and finalize recruitment and completed all study follow- ups. A total of 147 participants were randomized to treatment, despite significant COVID-19 challenges. Moreover, the team has completed and finalized all data entry and processing, while continuing regular data analysis activities. Regular team meetings are ongoing to plan for primary analyses as well as to discuss possible secondary papers.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: 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: Facilitating smoking cessation using reduced nicotine cigarettes: Intervention development and RCT study design.

Patents: None at the time of reporting.

Live Like Bella Initiative

Appendix Q

Fiscal Year 2021-2022 Newly Awarded Grants

Funded Fiscal Year 2021-2022

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
22L01	Baptist Health South Florida	Matthew Hall, MD	$ 250,000.00	3/31/24	No	No	No
22L02	The Nemours Foundation	Tamarah Westmoreland, MD, PhD	$ 250,000.00	3/31/25	No	No	No
22L03	University of Florida	Jonathan Licht, MD	$ 250,000.00	3/31/25	No	No	No
22L04	University of Central Florida	Alicja Copik, PhD	$ 250,000.00	3/31/25	No	No	No
22L05	University of Central Florida	Griffith Parks, PhD	$ 250,000.00	3/31/25	No	No	No
22L06	University of Florida	Loic Deleyrolle, PhD	$ 250,000.00	3/31/25	No	No	No
22L07	University of Florida	Paul Castillo Caro, MD	$ 250,000.00	3/31/25	No	No	Yes
22L08	University of Florida	Biljana Horn, MD	$ 899,897.00	3/31/26	No	No	No
22L09	University of Florida	Brian Stover, MD	$ 249,999.00	3/31/25	No	No	No
22L10	University of Florida	Ramzi Salloum, PhD	$ 100,104.00	3/31/24	No	Yes	No

1. **Grant #:** 22L01 Personalizing Radiotherapy Dose Using Genomic Markers of Radiosensitivity to Predict Tumor Response and Normal Tissue Toxicity in Pediatric Malignancies.

Principal Investigator: Matthew Hall, MD

Organization: Baptist Health South Florida

Abstract: Targeted therapies are increasingly used to personalize cancer care based on an individual patient's tumor genome. While radiotherapy (RT) technologies have advanced over time, patients largely receive the same empirical dose based on their diagnosis and the risk of normal tissue complications. To date, RT has not been adjusted based on inherent biological differences of patients or their tumors. In adults, the research team developed a radiosensitivity index (RSI) to stratify radiosensitive and radioresistant tumors and the genomic-adjusted radiation dose (GARD) to quantify the optimal RT dose for individual patients to achieve local disease control. RSI and GARD were studied in >20 adult tumor types including glioma, sarcoma, breast and lung cancers and were both prognostic and predictive of local tumor control and survival across multiple tumors. RSI and GARD were validated in adults but have never been studied in pediatric cancers. In this study, the research team aims to perform the first evaluation of RSI and GARD in childhood cancers. Pathology specimens from 200 pediatric and young adult patients treated with curative intent RT for tumors such as medulloblastoma, ependymoma, rhabdomyosarcoma, and Ewing sarcoma, will be collected. Tumor specimens will be assayed using a gene microarray. RSI and GARD will be calculated for each patient using the formula validated in adults. Using long-term follow-up data collected in survivorship clinics, the research team will evaluate whether RSI and GARD are correlated with local control, survival, and radiation-therapy toxicities.

The research protocol and the informed consent forms (ICF) are completed and approved. The study received IRB approval on 7/13/2022. If validated, RSI and GARD may enable oncologists to reduce RT dose for patients with radiosensitive tumors, which can maintain high cure rates while reducing the risk of late toxicities. In radioresistant tumors, clinicians may be able to dose escalate in selected patients to improve local control and survival. This could help to inaugurate personalized medicine in RT for the first time.

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 #:** 22L02 Treatment of Diffuse Intrinsic Pontine Glioma with the Oncolytic Zika Virus

Principal Investigator: Tamarah Westmoreland, MD, PhD

Organization: The Nemours Foundation

Abstract: The grant studying the treatment of diffuse intrinsic pontine glioma (DIPG) with the oncolytic Zika virus is starting well. The research team repeated the experiments confirming the sensitivity of DIPG to Zika virus. When exposed to a single dose of 1 million Zika viral particles, the DIPG cells demonstrate greater than 95% cell death. In addition to these experiments as planned in Aim 2 of the grant, the research team has determined that the DIPG cells activate the apoptosis pathway to die after exposure to Zika virus. This study will be repeated for confirmation. Furthermore, the cytotoxicity will be quantitated using an additional assay which will directly measure DIPG cell killing after exposure to Zika virus. Also, as described in Aim 2, attention was given to the importance of the cell surface marker, CD24, in Zika viral infections of DIPG. In neuroblastoma, CD24 is required for Zika viral sensitivity though the research team suspects that CD24 is not acting alone but is likely part of a complex. The research team found that CD24 is not expressed well in DIPG compared to a high risk neuroblastoma cell line. Even decreased levels of CD24 can influence Zika viral sensitivity; therefore, additional experiments to knock down the CD24 expression will be completed. More likely, CD24 is functioning in a complex that has other members which are significant in DIPG. As a result, the research team will be performing a proteomic study to determine the interacting partners of CD24. Additional DIPG cells are ready for in vitro studies to determine whether Zika virus produces a productive infection in DIPG patient samples. These studies are soon to be completed and will examine the protein Zika viral protein NS1 after exposure of the DIPG cells to Zika virus. If the DIPG cells have a productive infection, NS1 will be present. While these studies were being prepared, primers for the Zika envelope protein, which is located on the cell surface, were obtained and confirmed. These primers are required to complete the time course of viral genome quantity in infected DIPG cells. Parallel orthotopic murine studies are also underway to begin fulfilling Aim 1 of the grant. In these studies, the research team is injecting patient derived DIPG cells directly into the murine ventricle (brain). The initial round of injections was not successful, but the current ventricle injections are growing, and the mice are tolerating the procedure well. Overall, this grant has had a good beginning, and the experiments are falling into line to fulfill the overall goals of the grant.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

3. **Grant #:** 22L03 Elucidation and Targeting of Epigenetic Changes Resulting in Glucocorticoid Resistance in Pediatric Acute Lymphoblastic Leukemia

Principal Investigator: Jonathan Licht, MD

Organization: University of Florida

Abstract: Glucocorticoids (GC) are a major component of therapy of pediatric acute lymphoblastic leukemia (ALL). Relapse of ALL is associated with mutations/deletion of NR3C1 (glucocorticoid receptor, GR), NR3C2 (mineralocorticoid receptor- MR) and NSD2 (histone methyltransferase). These mutations may affect therapeutic response to GC. GR and MR both bind GC and enter the nucleus to alter gene expression. Both proteins may mediate the therapeutic effect of GC in ALL.

The Licht lab characterized a mutation of NSD2 (E1099K) in relapsed childhood ALL using cell lines and patient specimens. While NSD2 activated the expression of many genes it aberrant repressed the gene encoding for the glucocorticoid receptor leading to resistance of the cell to the killing effects of GC. Treatment of NSD2 mutant cell lines with EZH2 inhibitors, durgs that relieve the effects of a repressive protein on gene expression allowed the glucocorticoids receptor to again be activated by glucocorticoid treatment and allowed for killing of leukemia cells. Based on these findings a trial of EZH2 inhibitor and GC for relapsed ALL was proposed to the Children's Oncology Group. This research project will:

Aim 1: Determine how NSD2 mutations affect the phenotype and therapeutic response of ALL. The research staff will contrast the GC sensitivity, gene expression and epigenetic/chromatin changes driven by NSD2. This will be performed using isogenic gene edited cell lines and patient specimens. The research staff will determine how each of NSD2 mutations affect GR expression. Aim 2: Determine the role of MR in the response of ALL to GC The research staff will determine how GC response of ALL cells is affected by knockout of MR expression and determine binding of the MR in ALL. Aim 3: Develop methods to augment GC response of ALL. Since the grant has begun the lab explored how inhibitors of EZH2 could increase GC efficacy and found that there was synergy between GC and EZH2 inhibitors in cells without GR or MR mutations, but cells with such mutations were not sensitive to the two drug combination.

Follow on Funding: None at the time of reporting.

Collaborations: Richard Lock, PhD, Children's Cancer Institute Lowy Cancer Research Centre, UNSW Australia.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

4. **Grant #:** 22L04 Edited Natural Killer Cells As An Immunotherapeutic Approach for the Treatment of Pediatric Cancers

Principal Investigator: Alicja Copik, PhD

Organization: University of Central Florida

Abstract: Despite the advancements in treatment of pediatric tumors, for children with high-risk tumors, metastatic disease at diagnosis, or those with progressive, refractory, or relapsed disease only modest improvement in survival rates have been achieved. For example, the prognosis of children with high-risk neuroblastoma at diagnosis is less than 50%. There is a critical need for new treatment modalities that are effective in these high-risk patients. Immunotherapy has revolutionized treatment of advanced cancers in adults. In particular immunotherapy called checkpoint blockade, blocks receptors that prevent the immune system from attacking cancers cells. Blocking these negative regulators allows the body to better respond and kill cancer cells. Recently, there has been interest to apply immunotherapy in pediatric oncology. There are, however, several challenges to overcome before immunotherapy can become a standard of care for pediatric cancers. Unlike many adult cancers, most pediatric cancers are considered immunologically “cold.” This means there are not many immune cells present in and around the tumor, so it is difficult for the body to respond. This, combined with the fact that many of the current targets for blockade are not abundant in pediatric solid tumors makes it difficult for current immunotherapeutic strategies to be effective.

Natural Killer (NK) cells are an important part of the innate immune response. NK cells have an inherent ability to recognize and kill cancer cells and recruit other components of the immune system to further direct complete elimination of cancer. The researchers hypothesize that application of NK cells has the potential to turn "cold tumors", common in pediatric cancers, "hot" to greatly improve treatment outcomes. Recently, combination therapies using NK cells have shown antitumor responses against pediatric cancer cell types and have been used in a phase I clinical trial for treating younger patients with brain tumors. NK cell-based therapeutics can provide a viable solution to increase the success of immunotherapeutic strategies in pediatric cancers. This project aims to develop clinically translatable NK cell-based immunotherapeutic strategies that harness both the innate and adaptive immune response to increase the response rate and lower the relapse rate in pediatric cancer patients. All of the IRB and institutional approvals have been secured and the experimental work has been initiated.

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 #:** 22L05 Oncolytic Virus in Combination With NK Cells for Treatment Of Pediatric Cancers

Principal Investigator: Griffith Parks, PhD

Organization: University of Central Florida

Abstract: Pediatric cancers are a leading cause of death in children past infancy in the US, and it is estimated that world-wide a child is diagnosed with cancer every two minutes. There are major challenges in treatment of many pediatric cancers, since the cell biology and immunology of childhood cancers can differ substantially from adult cancers. This raises the important question of how to modify therapies such as those based on oncolytic (cancer lysing) viruses and immune-based therapies for more effective treatment of pediatric cancers. Natural killer

(NK) cells are an integral part of the innate immune system that play pivotal roles in clearance of tumor cells as well as viral infections. The research staff has developed a method for specific expansion of human NK cells that yields vastly superior NK cells, with approximately 10-fold to 100-fold higher cytotoxicity than NK cells generated with previous methods. Published work from the research staff has also shown that cancer cells infected with an oncolytic parainfluenza virus produce signals which enhance the ability of PM-21 NK cells to kill virus infected targets, but importantly these signals stimulate NK cells to also kill neighboring non-infected cancer cells. The research staff's oncolytic virus treatment can also modulate the surface of infected cancer cells to reduce expression of immune-suppressive molecules which can inhibit immune cell killing of cancer cells. Taken together, the long history of this research staff in defining unique properties of oncolytic virus and PM21 NK cells lead to testing the hypothesis that treatment of pediatric cancers would be greatly improved by combining these therapies. In this project, the research staff will utilize established approaches and reagents to test important pediatric tumor cell types for their ability to respond to single treatment or combined treatment with oncolytic virus and/or PM-21 NK cells. In the long term, results will provide a foundation of new information on novel treatments that can impact the burden of 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.

6. **Grant #:** 22L06 Co-opting TME Lactate Signal to Benefit T Cell Therapy

Principal Investigator: Loic Deleyrolle, PhD

Organization: University of Florida

Abstract: Complex alterations of energy pathways have been described in cancers and originate from the Warburg hypothesis, which postulates that the majority of cancer cells derive their energy from aerobic glycolysis. This specific metabolic reprogramming of strong engagement in the glycolytic pathway is a hallmark of high-grade glioma (HGG). As part of their high glycolytic rate, HGG secrete metabolic byproducts such as lactate, which is thought to act as an important oncometabolite and immunosuppressor.

It is now well recognized that HGG cell energetics strongly dictate the metabolic landscape of the tumor microenvironment (TME) supporting tumor development and growth. The TME is a complex network of diverse cellular compartments where tumor cells interact with a variety of non-neoplastic cells including immune cells, which represent key components of the tumor milieu. The metabolic specificities of HGG can determine fates and functions of neoplastic cells but also of immune cells creating specific niches, which play critical roles in restricting anti-tumor responses. Notwithstanding the presence of immune cells in HGG, the TME is globally immunosuppressive. Immune evasion and metabolic reprogramming are now well-recognized hallmarks of cancer and are considered to be functionally linked. Immune cells also possess defined metabolic characteristics and requirements and the tumor milieu metabolic status tightly controls the function of immune cells. Understanding and exploiting the mechanisms of these immunosuppressive metabolic conditions has promise for improving anti-tumor immunity and may help in developing novel immunotherapies. Specifically, lactate produced in the TME, as a

result of cancer cell metabolic rewiring, participates in immune escape via restricting T lymphocyte activity through the inhibition of their proliferation and cytokine production. Capitalizing on this current knowledge of tumor metabolism and how metabolic pathways affect immune response, this project proposes to test an innovative therapeutic modality based on reprograming the metabolic qualities of anti-tumor immune cells to enhance immunotherapy for the treatment of HGG. The research project staff hypothesize that co-opting lactate signal may be a useful approach to overcome metabolically driven tumor-imposed immunosuppression and for developing efficient immunotherapies. This project will test the efficacy of lactate receptor genetic engineering in T cells in the context of adoptive cell therapy to treat HGG.

The main innovation of this project is the integration of fundamental concepts of tumor and immune metabolism in the design of T cell therapy. The major impact of this study is that successfully completed it will demonstrate that immunometabolism represents a viable and critical target for the development of new cancer therapies to treat brain tumors, especially HGG and will validate a clinically applicable method to overcome treatment resistance to adoptive cellular therapy in 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.

7. **Grant #:** 22L07 Unlocking Car T Cell Efficacy Against Osteosarcoma Using Adjuvant R Vaccine

Principal Investigator: Paul Castillo Caro, MD

Organization: University of Florida

Abstract: The research team robustly confirmed the findings presented in the Live Like Bella grant proposal. A potent synergy between chimeric antigen receptor(CAR) T cells and RNA nanoparticles (RNA-NPs) was identified and was significantly better when nanoparticles were loaded with RNA encoding for the target antigen (i.e., CD70). Second, the research team was able to replace pre-CAR T cell conditioning (i.e., irradiation) by RNA-NPs under the premise that RNA-NPs induce enough systemic immune activation to create the appropriate environment for antitumor CAR T activity. This finding can potentially overcome the toxicities associated with chemotherapy/irradiation conditioning regimens prior to CAR T cells. Third, in in vitro studies it was shown that RNA-NPs can transduce surface antigen negative--tumor cells to express the antigen of interest and the transduced tumor cells were recognized and killed specifically by CAR T cells. Given these results, a survival experiment was performed where two immunocompetent murine models were intravenously injected with surface antigen negative-tumor cells (i.e., lung metastatic models of 816FO melanoma and K7M2 osteosarcoma). Interestingly, it was observed a significantly improved survival of tumor-bearing mice that received CAR T cells and RNA-NPs delivering the target antigen. Hence, the research team was able to transform a cold tumor into a hot tumor.

Currently, the research team is carrying out experiments to dissect the mechanistic underpinnings of this synergy. However, the other immediate goal is to translate this technology to a clinical trial in pet dogs with osteosarcoma before moving to a first-in-human clinical trial. In

mechanistic experiments, it was demonstrated that in the combination groups of CAR T cells and RNA-NPs, CAR T cells migrate to reticulo-endothelial system organs mainly spleen and interestingly, spleen-derived CAR T cells from mice treated with CAR T cells+ CD70RNA-NPs showed stronger antitumor activity. These findings suggest that RNA-NPs play a role in trafficking of CAR T cells and their killing activity is dependent on the RNA delivered by NPs. This approach might meet the urgent needs of the Floridian pediatric population with metastatic osteosarcoma that is associated with dismal prognosis without major improvements over the past three decades.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Nguyen DT, Ogando-Rivas E, Liu R, Wang T, Rubin J, Jin L, Tao H, Sawyer WW, Mendez-Gomez HR, Cascio M, Mitchell DA, Huang J, Sawyer WG, Sayour EJ, Castillo P. CAR T Cell Locomotion in Solid Tumor Microenvironment. Cells. 2022 Jun 20;11(12):1974.doi: 10.33

Patents: None at the time of reporting.

8. **Grant #:** 22L08 Engineered Donor Graft for Pediatric Hematopoietic Cell Transplant (HCT) Recipients with Hematologic Malignancies (HM) – Florida Pediatric Bone Marrow Transplant and Cell Therapy Consortium (FPBCC) First Prospective Multicenter Trial

Principal Investigator: Biljana Horn, MD

Organization: University of Florida

Abstract: In 2018, Florida pediatric bone marrow transplant (BMT) physicians from five institutions founded a consortium - Florida Pediatric BMT and Cell Therapy Consortium (FPBCC) with the goal of improving pediatric hematopoietic cell transplant (HCT) outcomes in Florida. FPBCC activities over the last three years have included sharing best practices during monthly consortium meetings, consortium-wide quality improvement projects, and extensive retrospective data analyses to identify priorities for prospective clinical trials. Improving survival rates of children undergoing HCT for hematologic malignancies (HM) was identified as the number one FPBCC priority.

Kaplan-Meier estimates of one-year and two-year overall survival (OS) of 150 children who received HCT for ALL and AML in FPBCC centers during the 2015-2019 period are 68% and 58% respectively. This low survival is due to a large proportion (65%) of high-risk patients, defined as those receiving human leukocyte antigens (HLA)-mismatched transplants and/or those with high-risk disease. One-year overall survival of high-risk patients was 58% and two-year survival was 50.5%. High-risk patients had high non-relapse (26%) and relapse (18%) mortality, at a median follow-up of 12 months. Improving survival, free of relapse and chronic graft-versus-host disease (cGVHD), in children receiving HCT for hematologic malignancy (HM) is the objective of the proposed multi-center trial. Graft engineering consists of precisely separating hematopoietic progenitor cells obtained by apheresis into components, e.g., stem cells, T-regulatory cells (Treg), and memory T-cells (Tmem), and then designing a graft with the optimal ratio of cell subsets. Pre-clinical and clinical research indicates that such engineered grafts result in reduced rates of GVHD, an undesirable complication of HCT, while maintaining anti-cancer immunity. Since the start of this award, the research project staff, in collaboration with the company (Orca Biosystems, Inc.) developed the first clinical trial which will study an

engineered donor graft (Orca-Q) in children receiving HCT for hematologic malignancies. Regulatory approvals (FDA approval) and institutional IRB approval at the leading institution have been secured. The trial is open for the enrollment at one institution currently and the trial will be rolled out at the other four Florida pediatric BMT programs. This novel and improved way of doing HCT will be available to the majority of Florida children getting HCT for hematologic malignancies.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

9. **Grant #:** 22L09 Ultrasound Elastogram Assessment of Liver Fibrosis in Children and Adolescents/Young Adults (Aya) Receiving Chemotherapy or Allogeneic Bone Marrow Transplantation, and Identification of Risk Factors for Liver Injury

Principal Investigator: Brian Stover, MD

Organization: University of Florida

Abstract: Survival rates for cancer have improved over the last several decades, but many of these cancer survivors are left with long term side effects from treatment. Cancer therapy is commonly associated with acute and often reversible hepatotoxicity, but there is little knowledge available regarding the long-term liver health of these patients. Current recommendations for follow-up of hepato-biliary late effects include annual evaluation of liver enzymes, bilirubin levels, and ferritin levels. This limited follow-up may underestimate the risk of chronic liver injury related to chemotherapy or other events during cancer treatment.

The objective of the study is to identify the incidence of liver fibrosis and/or liver cirrhosis using non-invasive ultrasound elastography in a population of children (12 years of age and older) and adolescent young adults (AYA) who received chemotherapy for treatment of cancer or during stem cell transplant. The hypothesis is that incidence of chemotherapy induced liver injury is higher than what is reported due to limited current followup. Risk factors for liver injury will be analyzed, and a potential relationship between polymorphism of genes involved in chemotherapy metabolism and the risk of liver injury will be explored. This is a cross sectional study which will enroll 100 children/AYA subjects who will have a liver ultrasound elastogram and will provide blood for pharmacogenomic testing. Detailed clinical data will be collected to gather information on risk factors for liver injury/fibrosis, as well as chemotherapy agents received to explore a relationship between genes involved in their metabolism and the risk of liver injury. The results of the liver elastogram will be shared with each subject and if the liver elastogram is abnormal, the subject will be referred to the gastroenterology service for further evaluation and treatment.

Results from this study will provide data for evidence-based guidelines that can be used in long-term follow-up of patients who received chemotherapy. Furthermore, identifying gene variants related to increased liver toxicity of chemotherapy agents will contribute to knowledge required for individualized patient treatment that will decrease organ injury risk.

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 #:** 22L10 Point-of-care Intervention to Address Financial Hardship in Families Facing Pediatric Cancer

Principal Investigator: Ramzi Salloum, PhD

Organization: University of Florida

Abstract: The purpose of this grant is to conduct a pragmatic trial evaluating the feasibility and acceptability of a financial counseling program for families facing pediatric cancer. This is a collaborative project conducted with the OneFlorida Clinical Research Consortium and Extension System, bringing together a collaborative team of investigators from the University of Florida (UF), University of South Florida (USF), and Tampa General Hospital (TGH).

Since the project start , approval from both the UF Institutional Review Board (IRB) and the UF Health Cancer Center's Scientific Review and Monitoring Committee was received. The research team has collaborated to outline a detailed recruitment protocol, which starts with an initial introduction of the study to the parent(s) with visually appealing flyers by either the patients' provider or social worker. Once the parent(s) express interest in learning more, the research staff, who are in embedded in the clinic/hospital, meet with the family to discuss the study and consent the parent(s). The research team just started recruitment in both inpatient and outpatient pediatric hematology/oncology settings at UF Health in Gainesville. Thus far, the research staff have enrolled one participant from UF.

At USF/TGH, the research staff are in the process of securing the USF IRB approval. The research team has prepared the protocol and visually appealing flyers for approval by the IRB. After the IRB application has been approved, the research team will start recruitment of parent(s) at TGH.

The research team has created a Research Electronic Data Capture (REDCap) database to capture the progression of participants through the study protocol, collection of parent-reported data (including the study outcomes of feasibility and acceptability of the financial counseling program), and financial counselor-provided information (including notes about the counseling sessions). By using this REDCap database, the study team will have the necessary infrastructure in place for the research coordinators to collaborate across the sites.

Follow on Funding: UF Health Cancer Center, Salloum and Moble, 4/1/2022-3/31/2024, Requested $55,712, In-kind donation to offset decrease from application budget to funded award, Funded: $55,712.

Collaborations: This study is a multidisciplinary collaborative effort across UF, USF, and TGH. The study is led by Drs. Ramzi Salloum and Erin Mobley (Co-Principal Investigators), both from the College of Medicine at UF. Dr. Salloum is an economist and implementation scientist in the Department of Health Outcomes and Biomedical Informatics in Gainesville. Dr. Mobley is a health services researcher in the Department of Surgery in Jacksonville. The Co-Investigators

on this project are located at UF and USF: At UF, Dr. Joanne Lagmay is a pediatric hematologist/oncologist in the Department of Pediatrics at the College of Medicine in Gainesville. Dr. Michael Gutter is the Associate Dean for Extension and is located in the Department of Family, Youth, and Community Sciences. Dr. Ji-Hyun Lee is a biostatistician located in the Department of Biostatistics in the College of Public Health and Health Professions and College of Medicine in Gainesville. Dr. Jennifer Brailsford has expertise in qualitative and mixed methods research and is located in the Center for Data Solutions at the College of Medicine in Jacksonville. Dr. Alex Parker is the Senior Associate Dean for Research in the College of Medicine in Jacksonville. At USF, Dr. Juan Rico is a practicing pediatric hematologist/oncologist in the Department of Pediatrics who sees patients at TGH. Research staff supported by this project are located at both UF and TGH: At UF, Dr. Anna Maria Abi Nehme serves as the study coordinator and oversees all study activities while collaborating with USF/TGH. At TGH, Ms. Alexa Steinbrueck serves as the study coordinator and oversees all study activities at USF/TGH in collaboration with Dr. Rico.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Live Like Bella Initiative
Appendix R
Fiscal Year 2021-2022 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	$800,990.00	4/30/26	No	No	No
21L03	University of Florida	Mingyi Xie, PhD	$247,000.00	4/30/24	Yes	No	No
21L04	H. Lee Moffitt Cancer Center and Research Institute	Uwe Rix, PhD	$247,000.00	6/30/24	No	No	No
21L05	University of Florida	Jonathan Licht, MD	$247,000.00	4/30/24	No	No	No
21L06	University of Florida	Lan B. Hoang-Minh, PhD	$247,000.00	4/30/24	No	No	No
21L07	University of Miami	Paulo S. Pinheiro, PhD	$247,000.00	4/30/24	No	No	No
21L08	University of Miami	Regina Graham, PhD	$247,000.00	6/30/23	No	Yes	No
21L09	University of Florida	Raymond Mailhot, MD, PhD	$247,000.00	4/30/24	No	No	No
21L10	Florida State University	Q.X. Amy Sang, PhD	$246,510.00	06/16/21	No	No	No
21L11	University of Central Florida	Annette R. Khaled, PhD	$123,500.00	07/01/21	No	Yes	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: The study team has been able to identify and onboard Greenlight Guru, a regulatory consulting firm that specializes in the preparation of investigational device exemption (IDE) submissions. Greenlight Guru is providing an online platform that facilitates the preparation of IDE submissions and provides critical regulatory input. Greenlight Guru provides expert input on all aspects of the IDE submission through regular meetings and access to document templates. The preparation of several clinical documents and SOPs has started. The researchers have started to identify normal tissue controls for the tissues of origins for different pediatric sarcoma subtypes. Additional samples are being identified and purchased moving forward. Appropriate suppliers have been identified and onboarded and will specifically identify tissue samples for the study team. The BioTek MultiFlo FX instrument has been purchased and installed. Experiments are currently ongoing for the integration and validation of the instrument.

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

Principal Investigator: Mingyi Xie, PhD

Organization: University of Florida

Abstract: The researchers are investigating the importance of a newly defined gene regulation mechanism, termed “target-directed microRNA degradation (TDMD)” in T-cell lymphoblastic leukemia (T-ALL). The researchers found a sequence in the 3' UTR of BCL2L11 mRNA can induce miR-221 and miR-222 degradation. Since BCL2L11 encoded protein BIM is a pro-apoptotic protein induced when T-ALL cells are treated with dexamethasone, and miR-221/222 are anti-apoptotic miRNAs, the cells probably cooperate to induce T-ALL apoptosis during dexamethasone treatment. Because BCL2L11 activation is a common feature in leukemia treatment, including both B-cell lymphoblastic leukemia (B-ALL) and T-ALL. The research team was wondering if the same TDMD phenomenon may present in other different cell lines. To this end, the team induced B-ALL cell lines 697 and SUP-B15 as well as an additional T-ALL cell line Molt4 with 1 μM dexamethasone. A control cell line K562 is also included in this experiment. Total RNAs were harvested from cells treated with dexamethasone or control vehicle. The induction of BCL2L11 mRNA was measured via reverse transcription followed by real time polymerase chain reaction (RT-qPCR) with two sets of primers targeting the coding sequence (CDS) and the TDMD region of the BCL2L11. Dexamethasone treatment induced at least a two-fold higher BCL2L11 expression in the B-ALL 697 and SUP-B15 cells. Subsequently, the researchers preformed northern blot analysis to measure levels of several microRNAs, including miR-221/222. Surprisingly, miR-221/222 levels increase in the B-ALL cell lines (Fig. B). This could be due to insufficient induction of BCL2L11 at only around two-fold. Similarly, control K562 and Molt4 cells did not show decrease in miR-221/222. The different responses of miR-221/222 to BCL2L11 induction will be of interest in the future studies as different cell lines/leukemia have different responsiveness to dexamethasone treatment. Elucidating the difference will help development of enhanced chemotherapy strategy by modulating the miRNA levels.

Follow on Funding: National Institute of General Medical Sciences, Mongyi Xie, 6/14/2022, Requested $60,771, Equipment Supplement for R35GM128753, Funded: $60,771

Collaborations: University of Florida is the major site for the proposed research. Tianqi Li, a fourth year Biomedical Sciences Ph.D. candidate and Nicholas Hiers, a second year Biomedical Sciences Ph.D. candidate are involved in the research.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Uwe Rix, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: Ewing's sarcoma (EWS) is a pediatric bone cancer that confers a dismal prognosis. There are no approved targeted therapies available for EWS, which would be more effective

and less toxic than the current chemotherapy regimens. Thus, EWS has a major need for novel therapies. Prior research by this laboratory has identified poly-ADP-ribosylpolymerase 16 (PARP16) as a new target in EWS, which was however sub-optimally targeted by existing inhibitors for the related protein PARP1. To develop better drugs for EWS patients that also target PARP16 or its downstream targets, it is critical to understand the biological mechanism of PARP16 in EWS cells and how it relates to the validated target PARP1 and the major EWS oncogene EWS-FLI1. This will allow development of appropriate functional readouts to measure drug impact on the cellular and molecular level. Specific Aim 1 of this project is to determine the cellular phenotypes affected by PARP16 and its in vivo functional relevance in EWS. Specific Aim 2 is to elucidate the molecular mechanisms that underlie the vulnerability of EWS cells to PARP16 targeting. During the first year of this project, work has been done to address both aims. Multiple genetic tools for silencing or overproducing PARP16 were assembled and validated in different EWS cell lines. In addition, two different EWS cell lines were engineered so that the PARP16 gene can be silenced by addition of the antibiotic doxycycline. This system can be used also in future mouse experiments to test the relevance of PARP16. Collectively, the different experiments confirmed the preliminary data that targeting of PARP16 in vitro reduces EWS cell viability and that this effect is further enhanced by treatment with PARP1 inhibitors, which are clinically approved for other cancers. Preliminary analyses suggest that these effects are likely independent of the chemotherapy-associated molecular marker SLFN11 and are elicited through a different cellular mechanism than apoptosis, which is a controlled form of cell death, whereas PARP1 inhibitor treatment does induce apoptosis. These results need to be further confirmed and expanded to additional EWS cell lines. In the meantime, quantitative mass spectrometry-based interrogation of the proteome indicated that doxycycline-mediated gene silencing of PARP16 reduces, as expected, PARP16 protein levels. In addition, it also prominently reduced protein levels of the important oncogene MYC, which is a known downstream target of EWS-FLI1. Consistently, several proteins that are known to be repressed by MYC activity were upregulated by PARP16 silencing. Notably, combined treatment with the PARP1 inhibitor olaparib further enhanced these effects. The effect of PARP16 silencing on MYC was independently confirmed also by immunoblotting. Collectively, these results suggest a functional effect of PARP16 on the EWS-FLI1/MYC axis, which is the major oncogenic driver pathway of EWS. This could indicate a new way how to target this pathway and provide a mechanistic rationale to develop urgently needed novel therapies for EWS patients, for instance by targeting both PARP16 and PARP1. Additional validation experiments are required and are ongoing.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Jonathan Licht, MD

Organization: University of Florida

Abstract: Major findings this past year regarding the mechanisms by which NSD2 a gene mutated and activated in relapsed childhood acute lymphoblastic leukemia (ALL) yields a more aggressive disease include:

The group published a paper in Cancer Discovery showing that NSD2 mutation caused glucocorticoid resistance in ALL. The lab found that genes consistently activated by mutant NSD2 across four cell lines analyzed lost the repressive H3K27me3 modification while three genes repressed in all of the cell lines gained the H3K27me3 modification. H3K36 methylation dramatically increased in intergenic regions including enhancer regions in NSD2 mutant ALL cells, preventing repressive EZH2/polycomb complex action. NSD2 mutation causes major changes in the three-dimensional configuration of chromatin in cells leading to more genes moving from inactive to active compartments of gene expression. The lab identified genes using gene editing screens responsible for the higher migratory activity of NSD2 mutant acute ALL which are undergoing validation as possible therapeutic targets. PTPRG identified was identifed as required for NSD2 mutant ALL cell migration in a gene editing screen. The activity was validated as depletion of PTPRG using RNA interference led to decreased migration and adhesion of NSD2 mutant ALL cells. This represents a potential new threapreutic target in ALL.The lab found that NSD2 mutation increased the ability of ALL cells to adhere to cell types that line the brain, consistent with their invasive activity in mouse models.

The lab identified genes induced upon exposure of ALL cells to cells of the lining of the brain that were involved in cell adhesion and signaling. Notably in NSD2 mutant ALL cells many of these genes were already increased in expression suggesting that NSD2 mutation preconditions ALL cells to invade the brain. FGF13 which is overexpressed in every cell line in which NSD2 is mutated in ALL (as well as in mantle cell lymphoma and multiple myeloma) acts as a protein preventing cell death.

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 #:** 21L06 Combination Immunotherapy for Pediatric Brain Tumors

Principal Investigator: Lan B. Hoang-Minh, PhD

Organization: University of Florida

Abstract: This study will significantly impact the health of Floridians by enhancing a promising therapy and significantly reducing the morbidity and mortality of pediatric patients with brain tumors and potentially other cancers. Malignant brain tumors are now the most frequent cause of pediatric cancer-related deaths. Despite aggressive and highly toxic therapies including surgery, radiation, and high-dose chemotherapy, almost half of the pediatric patients diagnosed with the most common malignant brain tumors, such as high-grade glioma and medulloblastoma, will die from recurrent disease. Survivors are often left with severe, lifelong treatment-associated neurological deficits and can develop secondary malignancies. 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 pediatric patients affected by

those aggressive cancers. Adoptive T cell therapy (ACT) involves using the patients' own immune cells, called T cells, to specifically kill their brain tumor. ACT has become the immunotherapy with the highest curative potential for patients with advanced cancers, 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 the research team's brain tumor 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 T cells. Immune checkpoint inhibitors against PD-1 have been used successfully in the clinic against multiple cancers, particularly when given before surgical tumor removal. Notably, recent early phase clinical trials have shown increased survival 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. The research team's studies show that anti-PD-1 treatment before surgery (neoadjuvant) increases the recruitment of T cells at recurrent tumor sites and enhances survival in a new preclinical resection model of recurrent glioma that the research team has established. The studies have found significant differences in immune exhaustion and activation pathway signaling, as well as gene expression, between neoadjuvant and adjuvant treatment groups. Particularly, cell cycle, IL-10, and TNF signaling pathways were upregulated in the neoadjuvant group when compared with the adjuvant group. Also, significant differences were found in the expression of immune checkpoint molecules on T cells. The proportion of T cells expressing those activation/exhaustion markers was increased in the neoadjuvant group. The team was also able to visualize ACT T cells over time after tagging the cells with optimized nanoparticles and using magnetic particle imaging, a new non-invasive imaging technology developed in collaboration with the University of Florida College of Engineering. These research findings have the high potential of being translated into novel, safer, and more effective immunotherapy approaches for pediatric brain tumors in the clinic, thus improving and extending or saving the lives of pediatric patients diagnosed with these very aggressive forms of cancer and potentially other types of 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.

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

Principal Investigator: Paulo S. Pinheiro, PhD

Organization: University of Miami

Abstract: The current study aims are to: characterize, for the first time, the incidence (risk) of childhood and adolescent cancers (i.e., acute lymphocytic leukemia, brain cancer and soft tissue sarcomas, among others) across a multitude of variables but with a focus on the detailed racial-ethnic backgrounds of children in Florida: White, African American, Afro-Caribbean, Haitian, Cuban, Mexican, Puerto Rican, Dominican, Central American, South American, Asian, and others; assess the role of potential exposures on cancer (e.g. diabetes and smoking) with a

focus on Hispanic and Black children; and assess survival disparities for the most common childhood cancers with the same focus on racial-ethnic disparities. This is a unique project to highlight distinctive risks that can be genetically related to specific childhood populations and disparities in cancer outcomes for childhood populations. The novel nature of this study is feasible in Florida given the size and unique diversity of the state's population.

This project relies on an essential linkage between Florida Cancer Data System (FCDS) data and Florida Department of Health (FDOH) Vital Statistics birth certificate data. Work on this study commenced as soon as the notice of award came through, and the data requests to Vital Statistics and FCDS were submitted promptly. The linkage is to be carried out by FCDS and FDOH according to the latest communication. Everyone has been diligent in this effort.

Follow on Funding: None at the time of reporting.

Collaborations: As this study is in the initial stages of preparing data requests and institutional review board applications, there is a planned collaboration between the University of Miami (UM)-Department of Public Health Sciences (Miami, FL) and University of Florida

Journals: None at the time of reporting.

Patents: None at the time of reporting.

7. **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: Brain tumors are now the leading cause of cancer-related death in children and such diagnoses are devastating for Floridian families. The goal of this research is to develop a novel nanomedicine which selectively targets brain tumor cells while also allowing for Magnetic Resonance Imaging (MRI). Direct imaging of the nanomedicine using MRI allows for non-invasive monitoring of drug delivery which will help guide therapy adjustments in real time. To create this nanomedicine, the research team is using carbon nitride dots (CNDs), a non-toxic nanomaterial, as the nanocarrier. Due to the high amounts of carboxyl and amino surface functional groups, the CNDs structurally mimic amino acids. Therefore, the working hypothesis is that by taking advantage of the increased demand for amino acids by cancer cells; developing a nanocarrier targeting amino acid transporters will increase tumor cell specificity and the efficacy of drug delivery. To facilitate MRI capability, gadolinium, an MRI imaging agent, will be incorporated into the CNDs. Aim 1 focuses on synthesizing, characterizing and optimizing a gadolinium CND derivative. While Aim 2 focuses on determining how surface functionalities influence the targeting and therapy of the CND against pediatric brain tumors.

Progress to date: Optimized CNDS: Original CNDs were synthesized with equal ratios (1:1) of citric acid (carbon source) and urea (nitrogen source). By increasing the ratio of urea to citric acid, the researchers have been able to synthesize new CNDs (1:3 and 1:5 ratios) that demonstrate improved cancer cell selectivity and increased drug loading capability. Furthermore, researchers determined that cell uptake of the CNDs is mediated by specific amino acid transporters known to be upregulated in cancer. Lastly, extensive physiochemical characterization of the CNDs suggests that modification of the CND surface functional groups

affect which transporter is used. This work opens the door for the developing personalized nanomedicines for specific cancers through modification of surface functional groups. Determined gadolinium source: Gadolinium-containing CNDs have been synthesized using Gadopentetic acid (GD-DTPA), brand name Magnevist which is currently used in the clinic, and gadolinium chloride (GdCL3). Characterization of Gadolinium CNDs (Gd-CNDs) revealed that GD-DTPA derived Gd-CNDs exhibit some toxicity and fail to retain their cancer cell selectivity. Conversely, GdCL3 derived Gd-CNDs are non-toxic and do retain cancer selectivity. The researchers previously demonstrated that attaching a peptide that targeted a receptor preferentially expressed on high-grade gliomas, increases entry of nanomedicine into the tumor cell and, therefore, tumor cell death. However, adding an additional peptide to enhance cell penetration and direct the nanomedicine to the cell nucleus dramatically increases the efficacy of the nanomedicine. Due to Florida Department of Health and Live Like Bella Cancer Initiative funding, this work has provided insight on how nanoparticles interact with tumor cells in order to increase the efficacy of future nanomedicines for the treatment of Floridians with cancer.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Kirbas Cilingir E, Sankaran M, Garber JM, Vallejo FA, Bartoli M, Tagliaferro A, Vanni, S Graham RM and Leblanc RM. Surface modification of carbon nitride dots by nanoarchitectonics for better drug loading and higher cancer selectivity. Nanoscale, 2022, 14, 9686-9701. DOI: 10.1039/d2nr02063g PMID: 35766148

Vallejo FA, Sanchez A, Cuglievan B, Walters WM, De Angulo G, Vanni S, and Graham RM. NAMPT Inhibition Induces Neuroblastoma Cell Death and Blocks Tu
mor Growth. Front. Oncol. 12:883318. DOI: 10.3389/fonc.2022.883318. PMCID: PMC9261286

Veliz EA, Kaplina A, Hettiarachchi SD, Yoham AL, Matta C,Safar S, Sankaran M, Abadi EL, Kirbas Cilingir E, Vallejo FA, Walters WM, Vanni S Leblanc RM and Graham RM.Chalcones as Anti-Glioblastoma Stem Cell Agent Alone or as Nanoparticle Formulation Using Carbon Dots as Nanocarrier. Pharmaceutics 2022, 14, 1465. https://doi.org/10.3390/ pharmaceutics14071465.

Paudyal S, Vallejo FA, Kirbas Cilingir E, Zhou Y, Mintz KJ, Pressman Y, Gu J, Vanni S, Graham RM, and Leblanc RM. DFMO Carbon Dots for Treatment of Neuroblastoma and Bioimaging. ACS Appl. Bio Mater. 2022, 5, 7, 3300–3309 Publication Date:June 30, 2022 https://doi.org/10.1021/acsabm.2c00309. PMID: 35771033

Mintz KJ, Kirbas Cilingir E, Nagaro G, Paudyal S, Zhou Y, Khadka D, Huang S, Graham RM, and Leblanc RM.Development of Red-Emissive Carbon Dots for Bioimaging through a Building Block Approach: Fundamental and Applied Studies. Bioconjugate Chem. 2022, 33, 1, 226–237 https://doi.org/10.1021/acs.bioconjchem.1c00544 PMID: 34914353

Patents: None at the time of reporting.

8. **Grant #:** 21L09 Measuring the Effects of Brain Radiotherapy on Scholastic Outcome

Principal Investigator: Raymond Mailhot, MD, PhD

Organization: University of Florida

Abstract: The purpose of this award is to understand the scholastic effects that radiotherapy has on young survivors of brain tumors. It has been demonstrated that IQ may decrease after brain radiation, but what is unclear is how school performance may change as this has never been evaluated in the United States.

This award seeks to marry two different datasets: educational data on annual statewide testing (Florida Standards Assessments) and the clinical information regarding the dose and location of the brain radiotherapy received. By combining that information, the researchers seek to evaluate the relationship between treatment and subsequent academic performance. The impact in successfully completing this award is manifold. This would be the first project in the USA to establish how IQ changes from radiotherapy manifest in school performance, and it would be the first to evaluate this scholastic effect in childhood recipients of proton therapy, a new type of brain radiation. This information would better provide Florida physicians and patient families with an understanding of how disease and treatment may influence academic performance, and it would serve as a foundation to explore this relationship on a national scale.

The project is complex given the data protections necessary to safely harbor student and patient information [Health Insurance Portability and Accountability Act (HIPAA) and Family Educational Rights and Privacy Act (FERPA), but with excellent staff coordination at both the Florida Department of Education (DOE) and the University of Florida, a consent was developed and approved. As such, the majority of this past year was safely developing an appropriate consent and establishing a partnership between the DOE and UF to partner the scholastic and medical information. With that protocol approved by both parties, consenting has started for a total of 44 childhood survivors at this current juncture. The researchers aim to complete accrual this upcoming year as well as begin data analysis.

Follow on Funding: None at the time of reporting.

Collaborations: The PI and his co-investigator team are all employed by the University of Florida. The team includes investigators from both the College of Medicine and the College of Education. This team includes Dr. Raymond Mailhot (PI) and Dr. Daniel Indelicato who are both radiation oncologists in the UF College of Medicine. Dr. David Miller is a co-investigator from the College of Education.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Q.X. Amy Sang, PhD

Organization: Florida State University

Abstract: A highly efficacious and specific therapy that maximizes the killing power against cancer cells and minimizes the adverse effects on normal cells do not exist for pediatric brain cancer patients. The human brain tumor microenvironment (TME) consists of cancer and noncancerous cells. TME heterogeneity can protect cancer cells from both the immune surveillance and therapeutic treatments. Hence, investigating the TME's effect on cancer cells and exploiting immunotherapies has become essential in brain cancer therapy. This project will build novel 3-dimensional (3-D) human pediatric brain cancer co-culture models using human

atypical teratoid/rhabdoid tumor (ATRT) cell lines and incorporating tumor microenvironment astrocytes and microglia. The hypothesis to be tested includes that tumor associated astrocytes may produce human interleukin-4 (IL-4) and other cytokines to polarize tumor associated microglia/macrophages. Natural killer (NK) cells and gamma delta-T (GD-T) cells are part of the innate immune system with anti-tumor activities. Both NK cells and GD-T cells will be tested for their ability to kill human pediatric brain cancer cells. To analyze the morphology of mature human astrocytes and the expression levels of astrocyte related biomarkers, normal human astrocyte cell line was cultured as a standard control. The research team has completed Specific Aim 1, Task 1, generating human astrocyte-like cells from hiPSCs, and Task 2, generating human microglia-like cells from hiPSCs. The research team members have started Aim 2 and completed hematopoietic differentiation and characterization. Researchers have further characterized hematopoietic progenitors for various biomarkers expression both quantitative and qualitatively using immunocytochemistry (ICC) and flow cytometry, respectively. The researchers have also successfully investigated differentiation of lymphoid progenitor stage, lymphoid stage and lineage towards innate immune cells such as NK cell and GD-T cells with the help of imaging approaches such as confocal and fluorescent microscopy and flow cytometry experiments.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Annette R. Khaled, PhD

Organization: University of Central Florida

Abstract: Cancer is the second leading cause of death in children, and the incidence of childhood cancers in Floridians is high. Cancers that occur in children are often diagnosed only when an abnormal lump is found by methods that can be invasive and damaging. To address the need for a cancer detection method that poses minimal harm to children, a liquid biopsy approach based on drawing a small amount of blood to detect circulating tumor cells (CTCs) is promising. However, unlike adult cancers, there are no liquid biopsy protocols approved for pediatric cancer detection. To address this unmet medical need, research staff developed a CTC detection protocol that centers on a novel biomarker, a protein-folding complex called CCT or Chaperonin-Containing TCP1. CCT is highly expressed in pediatric cancers, more so than adult cancers or normal tissues, and tumor tissues from pediatric cancers like rhabdomyosarcoma, glioblastoma, and neuroblastoma have very high levels of CCT, while normal tissues, such as healthy nerve or brain, have low levels. Expression of the chaperonin could thus be associated with invasive and deadly types of cancers. Using two cell lines from high-risk neuroblastoma patients to study the function of CCT in cancer, lab personnel engineered these cells to express more or less CCT. Addition of CCT resulted in more of the proteins that CCT folds, and neuroblastoma cells became larger and more invasive. When CCT was removed, the reverse happened. Neuroblastoma cells stopped moving or growing and died. CCT is thus essential for cancer cells, and its expression may identify CTCs with the potential to spread and destroy healthy organs. If CCT-high cancer cells are detected in blood, this could

alert doctors that their patients need more aggressive treatments. If CCT-low cells are detected in blood, this could indicate a milder form of cancer that could regress. To detect CCT-high neuroblastoma cells in blood, research staff modified the clinically approved CTC detection method, CellSearch, to incorporate the use of CCT as a marker for identification of neuroblastoma cells in blood. Success was shown by the ability to detect rare neuroblastoma cells amidst a background of thousands of blood cells, demonstrating that liquid biopsy for the detection of pediatric CTCs can be performed with a few teaspoons of blood. Outcomes from this research project were three manuscripts and a patent application filed for the use of CCT as a diagnostic marker for cancer.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Ghozlan, H, A. Cox, D. Nierenberg, S. King and A.Khaled.The TRiCky Business of Protein Folding in Health and Disease. Frontiers in Cell and Developmental Biology. May 5;10:906530. doi: 10.3389/fcell.2022.906530. eCollection 2022. (2) Cox A, Martin A

Patents: Detection of Chaperonin-Containing Tcp1(Cct) in Circulating Tumor Cells and Uses Thereof was filed on March 5, 2022 as a continuation-in-part (CIP) patent application.

Live Like Bella Initiative
Appendix S
Fiscal Year 2021-2022 Active Grants
Funded Fiscal Year 2019-2020

Grant #	Organization	Principal Investigator	Award Amount	End Date	Patents	Publications	Follow-on Funding
20L01	Florida State University	Akash Gunjan, PhD	$219,138.00	4/30/23	Yes	No	No
20L02	H. Lee Moffitt Cancer Center and Research Institute	Damon Reed, MD	$787,272.00	4/30/24	No	Yes	No
20L03	H. Lee Moffitt Cancer Center and Research Institute	Bijal Shah, MD, MS	$219,138.00	4/30/23	No	No	No
20L04	The Nemours Foundation	Tamarah Westmoreland, MD, PhD	$219,138.00	4/30/23	No	No	No
20L05	University of Central Florida	Cristina M. Fernandez-Valle, PhD	$218,572.00	7/1/23	No	No	No
20L06	University of Central Florida	Li-Mei Chen, MD, PhD	$109,569.00	5/31/23	Yes	No	No
20L07	University of Florida	Elias J. Sayour, MD, PhD	$788,897.00	6/30/23	No	No	No
20L08	University of Florida	Coy Heldermon, MD, PhD	$219,138.00	6/30/23	No	No	No
20L09	University of Miami	Julio Barrredo, MD	$219,138.00	11/30/22	No	No	No

1. **Grant #:** 20L01 Targeting wild-type Isocitrate Dehydrogenase (IDH) Enzymes for Treating Lethal Pediatric Diffuse Intrinsic Pontine Gliomas (Dipg) Driven by Histone H3.3 K27m Mutations

Principal Investigator: Akash Gunjan, PhD

Organization: Florida State University

Abstract: DNA is human genetic material, and it regulates all aspects of human health, including diseases such as cancer. Histones are proteins that bind the 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 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 H3.3K27M 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 over 80% of the 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 of DIPG tumors is mostly impossible. In addition, there are no approved chemotherapeutics to treat DIPG patients. DIPG's median age for diagnosis is six to seven years and the median survival rate is less than nine months following diagnosis. Hence, DIPG is currently a heartbreaking cancer for patients and their families, with no hope in sight.

Using patient derived tumor cells, the research staff has been studying the H3.3K27M mutant DIPG tumors with the goal of developing targeted therapeutics. So far, researchers have discovered that the H3.3K27M 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 (A-KG). High levels of A-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 H3.3K27M 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 H3.3K27M DIPG tumor cells, while mostly sparing the normal cells. A patent application was filed last year for this novel therapy. Since then, the research staff has prepared genomic sequencing libraries to understand the effects of IDH1 inhibitors at the molecular level. The research team is now preparing to test the effectiveness of this therapy in eradicating human 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: Florida Center for Brain Tumor Research, 9/1/22-8/31/23, $83,333, Pilot Award

Collaborations: Ernest O.N. Phillips, Sarah L. Menz, Serena Giovinazzi, Christopher Hagemeyer, Rakesh K. Singh, Daniele Canzani, Marie-Helene M. Kabbaj, Nicholas Leake, Aneesh Rahangdale, Michael W. Davidson, Jamila I. Horabin and Akash Gunjan. "Histone variant H3.3 play

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Damon Reed, MD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: The researchers have activated the trial here at Moffitt Cancer Center and have sent out information to all affiliate sites throughout the sunshine project network toward opening this study throughout Florida on this grant and throughout the nation with other foundation funds including from the National Pediatric Cancer Foundation. A total of 14 sites have been activated and are open to enrollment (three sites in Florida and 11 sites outside of Florida). Funds awarded are being allocated for the Florida sites only.

A total of four patients have been accrued on this multi-arm trial. Two patients enrolled on Arm A (first strike), one of which is currently active on study and one is on follow-up. Two patients enrolled on Arm B (maintenance), one of which is currently active and one is off study due to withdrawal during the follow-up period.

Follow on Funding: None at the time of reporting.

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

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 Mar 16.doi: 10.1002/cncr.32777. ePublication [ahead of print] PubMed PMID: 32176331.

March 10th webinar given through: UF Health Pediatric Hematology Oncology Educational Series. Title: Evolutionary Approaches to Therapy in Sarcoma, Speaker, Damon Reed

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 and Research Institute

Abstract: Pediatric Burkitt lymphoma (PEBL) is the most common (about 40%) non-Hodgkin lymphoma in children and adolescents in western countries. The prognosis of PEBL has steadily improved over the past 30 years with the introduction of highly intensive chemotherapeutic regimens with 80% five-year overall survival.

However, this success has come at the cost of significant toxicity secondary to intensive chemotherapy. Further, approximately 20% of patients with PEBL prove resistant to therapy or relapse soon after completing therapy. Outcomes in this group are dismal, with most succumbing to progressive lymphoma.

PEBL, and Burkitt lymphoma (BL) more broadly, are associated with a juxtaposition of the MYC gene, leading to its dysregulation and overexpression. The goal within this grant is to better understand how MYC protects PEBL cells from apoptosis, or 'programmed cell death', and develop interventions to target this.

In the previous update, the researchers described the dependence of PEBL on the anti-apoptotic protein MCL-1, and highlighted the relationship between MYC and the protein MCL-1. It was demonstrated that changes in MYC were associated with commensurate changes in MCL-1. It was further demonstrated that targeting MCL-1 directly could kill BL cell lines. Notably, the researchers also demonstrated that doxorubicin, a key chemotherapeutic component in PEBL and adult BL regimens, could act in part by suppressing MCL-1.

The research team identified BL cell lines with pre-existing resistance to MCL-1 inhibition, and similarly developed BL cell lines which developed MCL-1 resistance over time. Resistance to MCL-1 inhibition was associated with increased dependence on a different anti-apoptotic protein, BCL-XL. Looking cell signaling pathways that were increased in these resistant BL cells, the team identified an increase in STAT1 and STAT3 signaling as possible contributors to this altered survival protein dependence and resistance to therapy.

Progress over this past year has followed along these lines of investigation, as laboratory work was transitioned from Dr. Jianguo Tao (who departed Moffitt) to Dr. Bijal Shah and Dr. John Cleveland. Building on prior work, the researchers confirmed an increase in BCL-XL expression in the setting of enforced down regulation of MYC (ie, knockdown). However, an association with increases in STAT1 or STAT3 signaling was not seen. It was shown that targeting MYC differently with a drug that inhibits the interaction of all three major myc proteins (MYC, MYCN,

MYCL) with MAX blunted both MCL-1 and BCL-XL dependence to effectively kill BL. This was an unexpected observation. On further investigation, recently published work does identify a subset of PEBL which lack MYC dysregulation, and instead show dependence on MYCN.

Looking to public datasets, the researchers do in fact see MYCN expression across BL and BL-like lymphomas. Those lymphomas with gene expression more typical of BL interestingly show an increase in HRK and BIK – two important proteins which interact with BCL-XL to facilitate apoptosis. Unfortunately, there was no correlation between MYC and MYCN expression, making it difficult to elaborate on their individual contribution to cell survival. The researchers are working now to evaluate MYCN expression in the presence and absence of MCL-1 inhibition, to determine whether this may facilitate BCL-XL dependence.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Tamarah Westmoreland, MD, PhD

Organization: The Nemours Foundation

Abstract: Aim 1, which asses the cytotoxic activity of Zika viral treatment in cisplatin resistant CD24 positive high risk neuroblastoma cell lines, is complete. Within this aim, the research team created cisplatin resistant cell lines for paired neuroblastoma tumors that were pre and post treatment (recurrent). Following creation of these cell lines, the cell lines were then tested and confirmed to be resistant to cisplatin. The research team then grew the cells appropriately and exposed the cells to a single dose of Zika virus. Following this, greater than 95% of the cisplatin resistant neuroblastoma cells demonstrated cell killing four days after exposure, and all (100%) of the cells were killed by day five after Zika exposure. This was not dependent on whether the neuroblastoma cell line was pre or post chemotherapy treatment. The research group did note that the cisplatin resistant neuroblastoma cell lines had killing approximately 24 hours before the wild type, making one consider that the cisplatin resistance induced more sensitivity to Zika virus. Moreover, CD24, which is a cell surface marker in neuroblastoma, is required for Zika sensitivity in wild type neuoblastoma. The research team measured the expression of CD24 in the cisplatin resistant neuroblastoma cell lines to ensure that there was no change in the expression. Before and after exposure to Zika virus, CD24 was quantified in the cisplatin resistant paired neuroblastoma cell lines. There was no change in CD24 expression in any of the cell lines, which likely contributed to the Zika sensitivity of the cisplatin resistant cell lines. Currently, the research team is focused on Aim 2, which is the treatment of cisplatin resistant tumors grown in mice. The cisplatin resistant neuroblastoma cell lines were grown heterotopically on the hind leg of an immunocompromised mouse. The tumors are allowed to grow to 300 mm, and then Zika virus was directly injected into the tumor. This study is ongoing; however, the Zika treated tumors are responding to treatment. This study is a survival study to demonstrate a survival advantage to Zika viral therapy. Overall, this research demonstrating the utility of treating chemotherapy resistant neuroblastoma with Zika virus is a success. The

research team will continue the murine studies to complete Aim 2 and confirm in vivo treatment with Zika virus.

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 #:** 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: Children with Neurofibromatosis Type 1 who develop plexiform neurofibromas are at risk of developing malignant cells within the plexiform neurofibroma. The malignant cells known as malignant peripheral nerve sheath tumor cells are the primary reason for death of children with Neurofibromatosis Type 1. To date, there is no blood test to determine if a child at risk has developed a malignant tumor. Diagnosis is based on the appearance of pain, and rapid growth of a tumor shown on repeated magnetic resonance imaging that requires the child to be anesthetized for the procedure. The goal of this work is to adapt the Cell Search Circulating Tumor Cell Enumeration assay approved by the Food and Drug Administration for use as an early detection diagnostic method for malignant cells in children with Neurofibromatosis Type 1 who are at risk of developing a malignant tumor. The researchers have surveyed 11 human cell lines of malignant peripheral nerve sheath tumors (MPNST) and identified a consistently expressed receptor (epidermal growth factor receptor; EGFR) that may be useful in evaluating the presence of circulating MPNST cells in the blood of at risk patients. Ten of the 11 lines expressed this receptor at varying levels. The approved CellSearch assay however utilizes a different receptor (epidermal cell adhesion molecule; EpCAM) that the researchers found to not be as consistently expressed in the eleven model MPNST cell lines. Thus, a new EGFR dependent assay had to be established. The researchers are adding MPNST cells to normal blood samples and testing the ability to retrieve the cells from the blood using a commercial EGFR antibody. The retrieved cells are detected using flow cytometry analysis. The sensitivity of the assay appears to be in the hundred cell amount. Additional optimization of the antibody incubation, isolation and detections protocols are needed to increase the assay sensitivity to five cells or less. Once this is achieved, the researchers are ready to receive blood for patients diagnosed with MPNST.

Follow on Funding: None at the time of reporting.

Collaborations: University of Central Florida College of Medicine (Medical School Program) and the Burnett School of Biomedical Science (Graduate and Undergraduate programs) in Orlando FL., Parth Patel, Medical studentEthan Hass, MD/PhD student, Haley Hardin, Graduate student

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: This project is to investigate if matriptase protease can be activated in B cancer cells to limit and reduce the B cell lymphoma cancer progression.Matriptase is a serine protease that is over-expressed in B-cell lymphoma cancer cells and can promote cancer cell progression. Previous studies suggested that reducing matriptase in B cancer cells can limit cancer cell progression and tumor growth in an animal model. Matriptase is an enzyme that, once activated, can auto-cleave itself to produce more active enzyme. A method was developed to activate the matriptase in B cancer cells using exosomes as the vehicle. Exosomes are naturally occurring small vesicles produced by many cells. Exosomes are being explored as biomarkers and drug-delivery vehicles in disease diagnosis and treatment in recent years.

Non-Hodgkin lymphoma (NHL) is a type of cancer originating from cells that constitute the body's immune system. 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. Current treatments for NHL are mostly chemotherapy and surgery for early stages of the cancer. There is no standard treatment for chemo-resistant patients and the survival rate is at 10-30%, presenting an unmet challenge. 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 seven percentof all childhood cancers.

In the past year of this study, prostasin exosome-mediated matriptase activation in B cancer cells has been investigated. The activated matriptase activity was measured and confirmed, that matriptase was indeed being activated in B cancer cells with the introduction of prostasin exosomes. Several B cancer cell lines were recruited, cultured and treated with the exosomes. The data were analyzed to evaluate their cancer cell properties, including proliferation, migration, and invasion. The results are currently used for preparation of manuscripts for peer reviewed publications.

Follow on Funding: Becton, Dickinson and Company, Li-Mei Chen, MD, PhD, $5,000

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Elias J. Sayour, MD, PhD

Organization: University of Florida

Abstract: The project encountered significant and unanticipated hurdles outlined below in launching this first-in-human trial, using a first-in-class technology. The most significant of these has been the COVID-19 pandemic which created substantial delay (nearly a year) in initiating this complex study. There was a delay in purchasing equipment and onboarding new personnel to set up Qa/Qc workflows and manufacture product as dictated in the approved FDA-IND (BB-19304, Sayour). The team could not pursue validation of the technology due to backlogs on orders for vaccine ingredients. These ingredients were not received until March 2021, but validations have since taken place without significant complication. Institutional Review Board approval was approved in May 2021. There was also a change in the study monitor. Researchers must now identify an independent Data Safety and Monitoring Committee likely through a Contract Research Organization (CRO) to monitor this study. First, adult patients will be enrolled to the trial before enrolling pediatric patients (per FDA guidelines). The trial has now been activated and the research team is now screening and enrolling adult patients before researchers can begin the pediatric trial.

The research team has screened their first adult patient who is awaiting treatment expected to begin in March after chemoradiation. The researchers treated one adult patient with GBM using RNA-LP at a dose of 0.000625 mg/kg (0.039 mg of pp65 mRNA/tumor mRNA mixed 1:1). The patient received two doses before withdrawing from study citing desire to take a break from treatment and concerns regarding quality of life. The patient had seizure disorder from the underlying brain tumor treated with Keppra but developed breakthrough seizures four hours after the second vaccine. An adverse event was reported and attributed as unlikely related to study drug. The patient demonstrated immunologic response to vaccine based on margination and activation of peripheral blood mononuclear cells.

It is important to note that this project has already made a tremendous impact. The award has allowed creation of infrastructure for cGMP grade synthesis of mRNA vaccines, setup new quality assurance/release testing assays that have been approved by the FDA, and allowed creation of an immunomonitoring queue specific to mRNA cancer vaccines. This infrastructure can now be leveraged for conduct of new studies in pediatric cancer including recurrent pulmonary osteosarcoma.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Coy Heldermon, MD, PhD

Organization: University of Florida

Abstract: The purpose of the research is to find a new therapy for soft tissue sarcomas, a type of tumor that grows mainly in muscles, tendons and ligaments. This tumor type is very resistant to current therapies. Dr. Heldermon's Principal Investigator lab (PIL) used a mouse model that has mutations commonly found in cancers such as sarcoma. These mice develop cancers including sarcoma.

The PIL determined that some of the sarcomas that grew in these mice could in turn be given to other mice and will in turn grow, creating a new set of sarcoma mouse models call isograft sarcomas. The PIL also determined that some of these isografts could be dissociated to single cell suspensions and grow in subsequent mice enabling more consistent growth of the tumors. The PIL looked at the gene expression and sequences of some of the isograft sarcoma lines and determined that the isograft sarcoma lines are similar to the starting tumor and consistent with a human sarcoma.The PIL and the collaborating investigator, Dr. Sayour, isolated total tumor ribonucleic acid (TTRNA) and packaged this and control RNA (GFPRNA) into specially formulated lipid nanoparticles. The undissociated isograft sarcomas were grown in mice and the mice were treated with either naked nanoparticles, GFPRNA or TTRNA nanoparticles and both the RNA containing treatments slowed growth compared to naked nanoparticles but the growth was highly variable.

The experiment was repeated with the dissociated isograft sarcomas and the TTRNA nanoparticle treated mouse tumors grew statistically slower than the no RNA treated mice. The GFPRNA nanoparticle treated mice grew at a rate between the noRNA and TTRNA nanoparticle treated mice but did not reach statistical difference from either due to group size treated in this batch of the experiments. The next batch will increase the group size and allow more robust statistical comparison and confirm the effect of the treatment on immune activity. These results indicate that the TTRNA nanoparticle approach may be promising for the treatment of soft tissue sarcomas for Floridians.

Follow on Funding: None at the time of reporting.

Collaborations: University of Florida College of Medicine, Departments of Medicine, Pediatrics and Neurosurgery in Gainesville Florida were the sites of involvement.

Naddia Kabbej and Dominique Day were undergraduate researchers receiving training and performing research under this project.

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 Barrredo, MD

Organization: University of Miami

Abstract: This grant proposes to elucidate the mechanism(s) of epigenetic control of metabolic stress responses in ALL cells regulated by AMPK via interactions with chromatin-associated factors, and to exploit unique opportunities for translation of epigenetic-based therapies. Since the last legislative report, the research team has expanded the CHiP-Seq and RNA-Seq findings to include additional T-ALL (KE37) and B-LL cell lines (Kazumi 2, REH). The analysis has also been extended to include AMPKα1 and AMPKα2. These data, in addition to that generated in the CCRF-CEM cells (CN2) provide a comprehensive dataset for the analysis of the role of AMPK in altering gene expression in response to energy/metabolic stress. The researchers' analysis has been focused on the genes in which the team uncovered occupancy by AMPK at their transcription initiation sites. The Chip-Seq data indicate altered occupancy of AMPKα2

under conditions of energy/metabolic stress (glucose deprivation or AICAR treatment). The team confirmed this using CHiP-qPCR in Kazumi 2 and KE37 cells (that express high levels of AMPKα2) and found that histone genes' expression is decreased following AMPK activation. These findings were then extended and confirmed for AMPKα1 using NALM6, REH and CCRF-CEM cells. The researchers again found that occupancy by AMPK on histone genes' loci on chromatin decreases following AMPK activation, and correlates with decrease in histone genes' expression. As described in the proposal/preliminary data, using Co-IP experiments the researchers have uncovered that TAF1/7 interacts with AMPKα2 in the nucleus and likely directly on the AMPK/chromatin complex. Using computational prediction analysis (Scansite Motifs) of phosphorylation sites (site phosphorylated by serine/threonine or tyrosine kinases), it was identified that putative phosphorylated sites (p-sites) in the TAF1 protein that could be phosphorylated by AMPKα1/α2, a serine/threonine kinase. Further investigations using Co-IP experiments, kinase assays, genetic models and rescue experiments, confirmed the transcription factor TAF1 as a novel AMPKα2 partner and substrate in regulating histone genes' transcription. Mechanistically, using mass spectrometry the research team uncovered that AMPKα2 directly interacts with TAF1 at its N terminal domain; and phosphorylates TAF1 at Ser-1353 under cellular energy stress. To validate the mass spectrometry data and characterize the TAF1 Ser-1353 residue as the phosphorylation site in TAF1 targeted by AMPK, site-directed mutagenesis/gene editing (PCR mutagenesis) was used to generate the mutant TAF1 S1353A by substituting the serine 1353 residue with alanine (Ala),and compared its phosphorylation level to the TAF1 WT in HEK293 cells treated with energy/metabolic stressors. These findings were further confirmed using genetic constructs expressing constitutively active (CA) AMPKα2 (Flag-AMPK T172D) or inactive/dominant-negative (DN) AMPKα2 (HA-AMPK T172A) isoforms. More recent Chip-Seq-Seq data has identified AMPK occupancy on chromatin on cMyc's loci under energy stress conditions, and RNA-Seq showed decreased cMyc expression following AMPK activation and correlation with decreased expression of cMyc target genes. So far the researchers have uncovered a non-canonical function of AMPK that phosphorylates and regulates TAF1 on chromatin in response to energy stress.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Live Like Bella Initiative
Appendix T
Fiscal Year 2021-2022 Active Grants
Funded Fiscal Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
9LA02	H. Lee Moffitt Cancer Center and Research Institute	Mihaela Druta, MD	$784,733.00	3/31/23	No	No	No
9LA09	University of Miami	Julio Barredo, MD	$241,509.00	9/30/22	No	No	No

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

Principal Investigator: Mihaela Druta, MD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: The purpose of the study is to test 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, 2022, a total of 16 patients have been accrued (one replaced due to withdrawal prior to treatment and 1 will be replaced due to disease progression during their first week of treatment). The first six patients that were accrued on Dose Level 1 did not experience dose limiting toxicities (DLTs) 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 July 2020.

Dose Level 2 and 3 has a 3+3 study design. Based on how the first three patients perform on the trial will determine whether three more should be added at this same dose level. The researchers enrolled three patients on Dose Level 2, however, the third patient experienced a DLT (side effect) so three additional patients were added to Dose Level 2. No further DLTs were reported, so enrollment was opened on Dose Level 3. Three patients were enrolled on Dose level 3, however, the second patient experienced a DLT and the third patient had disease progression during their first week of treatment so will be replaced. How well the new third patient performs on Dose Level 3 will determine whether to add an additional three to Dose Level 3 or if the Maximum Tolerated Dose (MTD) has been reached. There is currently one active patient. All other patients are off treatment due to disease progression or withdrawal.

A total of 22 sites have been activated and are open to enrollment (five sites in Florida and 17 sites outside of Florida). During the last quarterly meeting with the project's Clinical Trials Oversight Committee on March 23, 2022, it was decided for the study to continue as designed (doctors not related to the Sunshine Trials review these trials and determine if the project can proceed or if there any red flags).

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

2. **Grant #:** 9LA09 Enhancing Immunotherapy Through Inhibition of Carbonic Anhydrase IX to Treat Osteosarcoma

Principal Investigator: Julio 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. The health care impact of this proposal is increasing the efficacy of immunotherapy for osteosarcoma (OS) patients. During this past year, Dr. Duan Zheng Fen, an expert in OS biology joined the project as a co-investigator. The research team initially used the intraosseous model proposed in the application based on implantation of K7M2-luciferase labeled cells in the tibia of BalbC mice. The team showed successful implantation of these cells in the intraosseous model and preliminarily in vivo treatment with the 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). In addition, the research team also optimized the humanized model to test this combination in primary human OS cells. 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. The early experiments showed the CAIX inhibitor WBI-5111 initially chosen to target CAIX did not inhibit growth and viability in OS cells unlike the prior experience with pancreatic cancer. On this basis, the team reasoned that a better experimental approach would be to knock down/out CAIX gene expression using either shRNA or CRISPR-Cas9, respectively. To that effect, during this last year efforts successfully knocked-down and knocked-out expression of CAIX in murine OS cells (K7M2, K12) using shRNA or CRISPR-Cas9, respectively. Successful knock-down/out was confirmed by immunofluorescence and Western blots comparing wild-type and transduced K7M2 and K12 under normoxia and hypoxia. Knock-down/out cells exhibited minimal or no expression of CAIX when compared to wild-type under hypoxia. Single cell clones were then selected and expanded to insure a uniform cell population in both cases. The research team's initial observation in these models with decreased or absent expression of CAIX was that cell growth (assessed by doubling time) under normal oxygen conditions (normoxia) was significantly slower that in wild-type cells. The team then proceeded to implant wild-type and CRISPR-CAS9 knock-out cells in the flanks of BALB/c mice and out of multiple single cell clones selected only clone H7 showed growth of knock-down cells in a few mice and after a prolonged period (30-45 days post-implantation). Repeat experiments failed to show tumor growth in BALBc mice following implantation of CAIX knock-down cells. Therefore, a conclusion of this work so far is that in OS cells, unlike other reported cancer cell models, complete knock-out of CAIX significantly impairs the ability of murine cells to grow in vivo. The research team has now optimized the use of shRNA to significantly, but not completely knock-down CAIX and assess the ability of these cells to grow tumors in mice, and if tumor formation is observed, the research team will use the shRNA model to test the combination of CAIX inhibition/downregulation plus PDL1 inhibition. Parallel, experiment are ongoing human OS cells to test their implantation on a humanized mouse model.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Live Like Bella Initiative
Appendix U
Fiscal Year 2021-2022 Active Grants
Funded Fiscal Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
8LA04	Baptist Health South Florida	Matthew D. Hall, MD, MBA	$700,000.00	10/30/22	Yes	Yes	No
8LA05	Florida International University	Diana Azzam, PhD	$700,000.00	3/10/22	No	Yes	Yes

1. **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: Baptist Health South Florida

Abstract: 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, researchers model early outcomes for change in hippocampal volume at six months following radiotherapy.

Follow on Funding: None at the time of reporting.

Collaborations: Nicklaus Children's Hospital, Miami, FL, Dr. Toba Niazi, Reshma Naidoo, PhD, Golnar Alamdari, PhD, Tampa General Hospital, Tampa, FL, Dr. Lawrence Berk, Florida International University, Miami, FL, Students

Journals: Hall MD, Naidoo R, Alamdari G, Niazi TN, Von Werne K, Rubens M, Kotecha R, Gallardo L, Yu J, Hobson M, Abrams K, Mohler A, Altman N, Medina S, Kalman NS, Chuong MD, Mehta MP. Change in Hippocampus Volume as a Function of Radiation Dose: Early Results from a Prospective Trial with Standardized Imaging and Morphometric Evaluation. International Journal of Radiation Oncology, Biology, Physics, Volume 111, Issue 3, e173 - e174.DOI: https://doi.org/10.1016/j.ijrobp.2021.07.659

Matthew Hall, Yazmin Odia, Katherine Von Werne, Toba Niazi, Ossama Maher, Ziad Khatib, Alexander Mohler, Rupesh Kotecha, Noah Kalman, Martin Tom, Kevin Abrams, Reshma Naidoo, Michael Chuong, Andrew Wroe, Alonso Gutierrez, Minesh Mehta, RONC-13. Change in hippocampus volume as a function of radiation dose: Results from a prospective trial with standardized imaging and morphometric evaluation, Neuro-Oncology, Volume 24, Issue Supplement_1, June 2022, Page i179, https://doi.org/10.1093/neuonc/noac079.667

Patents: None at the time of reporting.

2. **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: Pediatric cancers are fundamentally different from those in adults, with lower frequency of genetic mutations and fewer options for targeted therapies. The implementation of functional precision medicine (FPM) - the integration of ex vivo drug screening and mutation profiling- can, therefore, provide better treatment options for pediatric tumor patients. In this study, the research team investigated the feasibility and clinical utility of FPM in the management of pediatric patients with recurrent and/or refractory cancers. The team used a functional ex vivo 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 scores (DSS) were calculated for each drug based on cancer cells' responses. DST results were then combined with results from targeted mutation profiles to match actionable mutations with selective targeted therapies. The research team recruited a total of 25 patients into this ongoing clinical trial (number NCT03860376) and were able to perform drug testing and mutation profiling on 21 patients. The team optimized and successfully performed DST on at least 13 different tumor types including acute myeloid leukemia, chronic lymphoblastic leukemia, ependymoma, osteosarcoma, Ewing's sarcoma, rhabdomyosarcoma, glioblastoma, medulloblastoma, astrocytoma, neuroblastoma, rhabdoid, lung, and liver tumors. The feasibility study, so far, has demonstrated that ex vivo DST can be performed within a clinically actionable timeframe (median: seven days). Ex vivo DST returned between 10 and 30 treatment 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. Remarkably, DST provided valuable information to the oncologists on drug dosing and treatments that may not be effective and should be avoided. DSS synergizes with genomic data to further refine treatment recommendations. FPM-guided treatment regimens resulted in encouraging partial and complete responses as compared to progressive disease in prior regimens and physician choice regimens. Thus, this study shows technical feasibility of integrating functional precision medicine approaches for patients with refractory/relapsed pediatric cancers. Routine clinical integration of FPM for treatment selection is technically feasible and has led to improved treatment of pediatric cancer patients with refractory malignancies in an initial patient cohort, warranting further investigation.

   Follow on Funding: None at the time of reporting.

   Collaborations: None at the time of reporting.

   Journals: Acanda De La Rocha AM, Fader M, Coats ER, Espinal PS, Berrios V, Saghira C, Sotto I, Shakya R, Janvier M, Khatib Z, Abdella H, Bittle M, Andrade-Feraud CM, Guilarte TR, McCafferty-Fernandez J, Salyakina D, Azzam DJ. Clinical Utility of Functional Precision Medicine in the Management of Recurrent/Relapsed Childhood Rhabdomyosarcoma. JCO Precis Oncol. 2021 Oct 27; 5:PO.20.00438. doi:10.1200/PO.20.00438. PMID: 34738048; PMCID: PMC8563073.

   Patents: None at the time of reporting.

Live Like Bella Initiative
Appendix v
Fiscal Year 2021-2022 Closed Grants
Funded Fiscal Year 2020-2021

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
21L02	University of Florida	Zhijian Qian PhD	$100,000.00	10/31/21	No	No	No

1. **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 patents, 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-rAMLs. 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 research seeks to develop a novel strategy for a more effective treatment of MLL-r AMLs by selectively targeting the LSCs. Foxhead box protein M1 (FOXM1) is a critical gene which regulates many biological processes in the mammalian cells. It was found that FOXM1 was upregulated in MLL-rAML 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). This study investigates how Foxm1 upregulated on 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, FOXM1 was evaluated as a potential therapeutic target in the LSCs for pediatric MLL-r AML patients. This study advances the understanding of the molecular biology of LSCs and will likely lead to the development of novel therapeutics. Funding for this grant is bridge funding which supports an additional six months on the research project generated additional preliminary data to strengthen future federal grant applications. Progress has been made during this period. Interesting data was collected, which further supports the hypothesis for future RO1 grant application.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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Appendix W

Fiscal Year 2021-2022 Closed Grants

Funded Fiscal Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
9LA01	Florida State University	Q.X. Amy Sang, PhD	$250,000.00	3/31/22	No	No	No
9LA03	H. Lee Moffitt Cancer Center and Research Institute	Keiran Smalley, PhD	$250,000.00	3/31/22	No	Yes	Yes
9LA04	University of Florida	Jatinder Lamba, PhD	$223,758.00	3/31/22	No	No	No
9LA05	University of Florida	Zhijian Qian, PhD	$250,000.00	3/31/22	No	No	No
9LA06	University of Miami	Rosemeire Yanashiro-Takeuchi, DVM, PhD formerly Claudia Rodrigues, PhD	$250,000.00	3/31/22	No	Yes	No
9LA07	University of Miami	Anthony Capobianco, PhD	$250,000.00	4/31/2022	No	No	Yes
9LA08	University of Miami	Alan Pollack, MD, PhD	$250,000.00	3/31/22	No	Yes	Yes
9LA10	University of South Florida	Mildred Acevedo-Duncan, PhD	$250,000.00	4/30/22	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 the research team using induced pluripotent stem cell (iPSC) lines [Hua et al., Tissue Eng Part A. 2021 Jul;27(13-14):881-893]. This funded project has built 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 were utilized to generate this novel human pediatric brain cancer model for 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.

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 #:** 9LA03 Defining and Modeling Pediatric Melanoma Development

Principal Investigator: Keiran Smalley, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract: The single cell analyses of the mouse skin were the first of its kind, and a number of key observations were made. First, key differences were noted in the immune environment of neonatal skin, which was characterized by high levels of mast cells, T helper type 2 (Th2) polarized T cells and gamma delta T cells. Following UVR treatment, neonatal mice exhibited little infiltrate of cytotoxic T cells but were infiltrated by large numbers of mast cells. Later responses to UVR were characterized by gamma delta T cell infiltrate. By contrast, adult skin was characterized by an acute influx of macrophages and a delayed cytotoxic T cell response. These findings have been confirmed through IHC and flow cytometry analysis.

The study pathologist Dr. Messina has collected and annotated a large cohort of this rare subtype of melanoma. We currently have over 30 samples of pediatric melanoma from a wide age range (1-18 years of age). These samples will be processed and sent for targeted exome sequencing in the near future. Dr. Karreth and his team have been working to determine the best genetic background for UV-induced melanoma development in the neonatal setting. As the data from the human clinical specimens is still undergoing analysis, the group has focused upon the comparison of the BRAF V600E/Cdkn2a model and the more commonly used BRAF V600E/PTEN silencing models. A range of UV irradiation schedules have been explored, including modulating the doses of UVR and the timing of UVR treatment. The analysis of the BRAF/Cdkn2a cohort has been completed, and the analysis of the BRAF/PTEN model is still ongoing. These studies were delayed due to COVID shut downs (no animal experiments were permitted for some time). As these are long term studies (typically 1 year long experiments), completion has been delayed.

During the funding period we devoted considerable effort to developing our bioinformatic infrastructure to map cell-cell communication between mouse tumor cells and immune cells from our samples of irradiated skin/tumors. Our new platforms are based on two new tools, CellPhoneDB and SingleCellSignalR, which we have utilized to analyze clinical specimens of acral melanoma and mouse melanomas.

Follow on Funding: NIH/NCI, 01/01/2022-12/31/2023, Funded in the amount of $433,255

Collaborations: None at the time of reporting.

Journals: Li, J., Smalley, I., Chen, Z., Wu, J., Phadke, M., Teer, J., Karreth, F., Koomen, J., Sarnaik, A.A., Sondak, V., Zager, J.S., Khushalani, N., Tarhini, A.A., Rodriguez, P., Messina, J.L., Chen, A., Smalley, K.S.M, Single cell characterization of the Immune Environment of Acral Melanoma Identifies Novel Targets for Immunotherapy.

Patents: None at the time of reporting.

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

Principal Investigator: Jatinder Lamba, PhD

Organization: University of Florida

Abstract: This study has identified SNPs in the pharmamcology genes predicted interpatient variability in BuFluTT PK drug profiles and one-month donor chimerism. Results so far showed potential to predict outcomes and develop strategies that will consider pharmacogenomics when determining fludarabine doses in pediatric HCT recipients. This ongoing study is focused on

establishing and validating the pharmacogenetic markers predictive of pharmacokinetics of busulfan, fludarabine and thiotepa and clinical outcomes in pediatric HCT recipients.

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

Principal Investigator: Zhijian Qian, PhD

Organization: University of Florida

Abstract: Pediatric Acute Myeloid Leukemia (AML), which is a cancer of myeloid line of blood cells, represents 1520% of all pediatric acute leukemia. Ectopic Viral Integration site 1 (EVI1) is a known oncogenic gene that cause cancer when it is aberrantly expressed in cells. EVI1 upregulation is implicated in 1025% of primary AML in children and young adults and has an inferior outcome with current chemotherapy regimens. However, hypomethylating agents are not currently used as standard chemotherapy for upfront treatment of AML in children and young adults. Their use is limited to the relapse setting where certain AML patients respond to them. It is not well understood what determines response to these drugs. Researchers recently demonstrated a novel role of EVI1 as an epigenetic regulator in hematopoietic progenitor cells and found that high EVI1 expression leads to global changes in DNA methylation in human CD34+ stem/progenitor cells. In addition, hyperexpression of EVI1 led to promoter hypermethylation and downregulation of critical genes involved in cell proliferation and differentiation in leukemia cells. More importantly, reversing DNA hypermethylation by hypomethylating agents of the gene silenced by EVI1 inhibited growth and induced apoptosis of EVI1high human leukemia cells but not the leukemia cells with low
EVI1 expression. To explore the role of EVI1 in hematopoietic stem/progenitor cells in vivo, researchers have established a novel mouse model in which EVI1 hyperexpression can be induced specifically in hematopoietic stem/progenitor cells. By using this newly established mouse model, human leukemia cell lines as well as primary leukemia cells from pediatric patients, researchers will determine the role of the critical downstream pathways that are deregulated by EVI1 because of EVI1induced DNA hypermethylation in the development of leukemia and determine whether and how hypomethylating agents work for the subgroup of EVI1high pediatric AML. These studies advance the understanding of the biology of EVI1high pediatric AML and provide new molecular insights into therapeutic effects of hypomethylating agents in the subgroup of EVI1high pediatric AML.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Rosemeire Yanashiro-Takeuchi, DVM, PhD formerly Claudia Rodrigues, PhD

Organization: University of Miami

Abstract: Endothelial cell death from doxorubicin exposure may lead to micro-ischemic areas in the heart and may lead to myocyte death. Revascularization through activation of local endothelial cells should limit the degree of injury. However, development of senescence impairs revascularization, further contributing to myocyte death. Senescent cells release soluble mediators that will activate other cell types (inflammatory cells and fibroblasts) to repair the injured tissue. However, deregulation of these mechanisms may ultimately lead to mal- adaptive cardiac remodeling. The goal of this Aim is to identify endothelial-associated mechanisms that contribute to anthracycline-induced cardiac damage and progressive remodeling changes that lead to heart failure.

The research team has been able to finalize all the PV-loop data analysis and molecular data analysis pending during this final period. Hemodynamics studies were performed at endpoint, 12-months post-exposure to doxorubicin. Results show that males exposed to doxorubicin develop significant contractility changes as indicated by increase in the maximum rate of left ventricular (LV) pressure rise to the end-diastolic volume (dP/dTmax EDV). Interestingly, it was found that females exposed to doxorubicin show signs of contractile dysfunction as indicated by an increase in the pressure-volume area/end-systolic volume ratio (PVA/ESV), while no significant changes were found in males. In summary, our results show significant differences between male and female mice exposed to doxorubicin. Sex-related differences have also been described in patients, suggesting that different mechanisms are likely involved in doxorubicin cardiotoxicity. Dr. Rodrigues has also finalized the RNA sequencing analysis as part of the subcontract agreement created with Florida Atlantic University after her departure from the University of Miami.

As previously reported the Principal Investigator, Dr. Rodrigues, transferred to Florida Atlantic University (FAU). As per the Florida Department of Health (FLDOH) regulations, Dr. Rodrigues was not able to carry the award to FAU with her. A decision was made to assign a new principal investigator at the University of Miami (UM) and the implementation of a subcontract between FAU and UM. This process was initiated on June 2021, but was only completed on December 2021.

Follow on Funding: FHRF, 8/2/2021-7/31/2022, $212,437.50

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

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, researchers proposed to identify and target components of signaling networks that regulate TRP53 SHH MB viability. The 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.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Fan Yang, Jezabel Rodriguez-Blanco, Jun Long, Marzena Swiderska-Syn,Daniel T. Wynn,Bin Li, Chen Shen, Yuguang Ban, Xiaodian Sun, Robert K. Suter, Heather J. McCrea, Anthony J. Capobianco, Nagi G. Ayad, and David J. Robbins, A druggable UHRF1/DNMT1/GLIc

Patents: None at the time of reporting.

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

Principal Investigator: Alan Pollack, MD, PhD

Organization: University of Miami

Abstract: The long-term goal of the research project staff is to discover a molecular-based protective or mitigating strategy for radiation nephrotoxicity (RN). The objective was to investigate the mechanistic role of Sphingomyelin Phosphodiesterase Acid Like 3B (SMPDL3b) in renal injury after a single dose and fractionated total body irradiation (TBI). Research staff used SMPDL3b wild type (SMP-WT), SMPDL3b knockout (SMP-KO), and SMPDL3b overexpressing (SMP-OE) human podocyte and podocyte-specific SMP-KO adult and young mice to investigate the role of SMPDL3b in RN.

As proposed in AIM 1, research staff has established that radiation induces podocyte injury and reduces slit diaphragm (SD) protein nephrin expression. Radiation therapy (RT) causes podocyte injury and increases podocyte loss. The glomerular basement membrane (GBM) thickness and renal fibrosis increased, while renal function decreased in a dose and time-dependent manner. Altogether, these changes cause GBM thickness and albuminuria. These findings suggest that RT-induced GBM thickness and renal dysfunction in the experimental RN model depend on the podocytes' disarrangement and detachment, which might change the charge and size selectivity of the GBM, causing albuminuria. Research staff also established that TBI plus bone marrow transplantation combined with CD4+ plus CD8+ T-cell treatment has a more severe immune response than TBI alone and causes more renal damage after TBI. These experiments were part of the design to determine the effects of bone marrow transplantation and graft versus host disease (GVHD) on the renal injury.

As proposed in AIM 1.1, research staff has established that RT-induced SMPDL3b deficiency makes podocytes more susceptible to DNA damage and apoptosis. Also, research staff has established that SMPDL3b overexpression enhanced double-strand DNA breaks (DSBs) recognition and repair through the modulation of Ataxia telangiectasia mutated (ATM) nuclear shuttling. OE podocytes were protected against radiation-induced apoptosis by increasing the phosphorylation of p53 at serine 15 and attenuating subsequent caspase-3 cleavage.

As proposed in AIM 2, research staff has established that SMPDL3b overexpression prevented radiation-induced alterations in nuclear ceramide-1-phosphate (C1P) and ceramide levels. Interestingly, exogenous C1P pretreatment radiosensitized OE podocytes by delaying ATM nuclear foci formation and DSBs repair. Therefore, these results unravel a novel role for SMPDL3b in radiation-induced DNA damage response. The current work suggests that SMPDL3b modulates nuclear sphingolipid metabolism, ATM nuclear shuttling, and DSBs repair.

The proposed research significantly impacts Floridians' health because it describes SMPDL3b as a novel target of radiation-induced renal injury. It is possible that targeting SMPDL3b may represent a new therapeutic approach to prevent radiation-induced renal damage 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. Fractionated TBI is an effective conditioning regimen for hematopoietic stem cell transplantation. However, this is associated with acute and chronic adverse normal tissue toxicities. This study directly addresses aspects of reducing renal toxicity. This project will benefit Floridians by reducing mortality and morbidity, resulting in a significant health benefit for this patient population.

Follow on Funding: Department of Defense, Anis Ahmad, 10/1/2022-9/30/23, $115,125

Collaborations: None at the time of 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 January 19;1-31. PMID: 33464992.

Marina Francis, Larry Bodgi, Anis Ahmad, Patrick Azzam, Tarek Youssef, Alaa Abou Daher M.S1, Assaad A. Eid DSc1, Alessia Fornoni, Alan Pollack, Brian Marples, and Youssef H. Zeidan. SMPDL3b Modulates Radiation-Induced DNA Damage Response in Renal Podocytes. FASEB (Manuscript # 202100186). DOI: 10.1096/fj.202100186RR.

Anis Ahmad, Jumana Afaghani, Saba Ansari, Sandra Merscher, Youssef Zeidan, Alan Pollack, Alessia Fornoni, Brian Marples*. Mechanism of radiation induced glomerular basement membrane thickness. Matrix Biology (Manuscript # MATBIO-D-22-00200).

Patents: None at the time of reporting.

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

Principal Investigator: Mildred Acevedo-Duncan, PhD

Organization: University of South Florida

Abstract: Neuroblastoma (NB) is the pediatric cancer of the early nerve cells in which solid tumors originate from the peripheral sympathetic nervous system and as such can occur primarily anywhere along the sympathetic chain with development on the adrenal gland being most common. The expression of the disease is highly variable as in some cases lead to spontaneously regression without any medical intervention whereas in other cases the tumors become highly metastatic and therapeutically resistant. The results demonstrated that atypical protein kinase C-iota and zeta (aPKC-ι/ζ) levels were overexpressed in NB cells (BE-2C and BE-M17) and tissues.

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 Initiative
Appendix x
Fiscal Year 2021-2022 Closed Grants
Funded Fiscal Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
8LA02	University of Central Florida	Cristina Fernandez-Valle, PhD	$200,000.00	10/31/21	No	No	No

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

Principal Investigator: Cristina Fernandez-Valle, PhD

Organization: University of Central Florida

Abstract: Researchers completed the immunohistochemical analysis of allografts from the in vivo study. Researchers prepared slides with sections from four allograft tumors for each of the four groups to increase confidence in the findings and performed the H&E, S100, pPAK and pAKT; additionally immunostained for Ki67 and cleaved caspase 3 immunohistochemistry. These important protein markers were quantified to allow for direct comparison in expression across each treatment group.

Follow on Funding: None at the time of reporting.

Collaborations: Four undergraduate students and one medical student at the University of Central Florida in the Burnett School of Biomedical Sciences, College of Medicine are training and contributing to this project.

Journals: None at the time of reporting.

Patents: None at the time of reporting.