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

2022-2023 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 (Live Like Bella) (both found in s. 381.922, Florida Statutes) and the James and Esther King Biomedical Research Program (King Program) (s. 215.5602, Florida Statutes). During Fiscal Year (FY) 2022-23, $18,612,434 was awarded to Bankhead-Coley Program, Live Like Bella, and King Program grantees. FY 2022-23 funding provided for 16 Bankhead-Coley, 13 Live Like Bella and nine King Program new research grants made to universities and cancer research centers across the state to support researchers working to improve cancer prevention, diagnosis, and treatment.

Biomedical research grants are awarded through a competitive peer review process. Awards are based on scientific merit, as determined by independent peer review by experts who are (1) located outside Florida and (2) free from conflicts of interest. Full-time researchers at any Florida-based university or established research institution are eligible to apply. All awardees provide regular updates that are included in this annual report. In addition, a Long-Term Impact Survey was conducted in 2023 and Biomedical Research grant recipients from 2012-2014 were asked to identify the long-term impacts resulting from the research studies. Details are found in Appendix A.

Per statutory requirements, the progress reports from the grantees includes the following information:

- Florida’s rank relative to other states along with its total National Institutes of Health (NIH) biomedical research funding.

- 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 Program advances progress toward cures for cancer. Cancer is the second leading cause of death for Floridians, after heart disease. Funding through this program aims to improve 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 Initiative is to advance progress toward curing pediatric cancer through grants awarded through a peer-reviewed, competitive process. The Live Like Bella Initiative will provide grants for research to further the search for cures for pediatric cancer, by pursuing the following goals:

- Expanding pediatric cancer research capacity in Florida.
- Improving both research and treatment through greater pediatric enrollment in clinical trial networks.
- Reducing the impact of pediatric cancer on disparate groups.

James and Esther King Biomedical Research Program

The purpose of the King 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 long-term goals of the program are:

- Improving the health of Floridians by researching better prevention, diagnoses, treatments, and cures for cancer, cardiovascular disease, stroke, and pulmonary disease.
- Expanding 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.

- Improving 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.

- Increasing 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.

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

Cancer-Focused Research Funding, 2012-2023

The Florida Department of Health (Department) Biomedical Research Division reviewed all the Biomedical Research Grant Programs to determine the types of cancer research were funded from 2012-2023. All grants awarded were recorded and categorized based on focus and research area. Results of this review found the following:

- Since 2012, the Department has awarded a total of 389 grants for research of various cancers and tobacco-related diseases.

- Across all cancer research studies, breast cancer was the most prevalent award focus with lung cancer following closely behind. See Exhibit 1 below.

- The cancer types with the fewest grant research studies included kidney cancer, cervical cancer, sarcoma, and colon cancer.

In addition to research focused on specific cancers, grant funding also supports the creation of new technology, treatments, and interventions in the fight against cancer as well as projects related to tobacco, nicotine, and smoking in general.

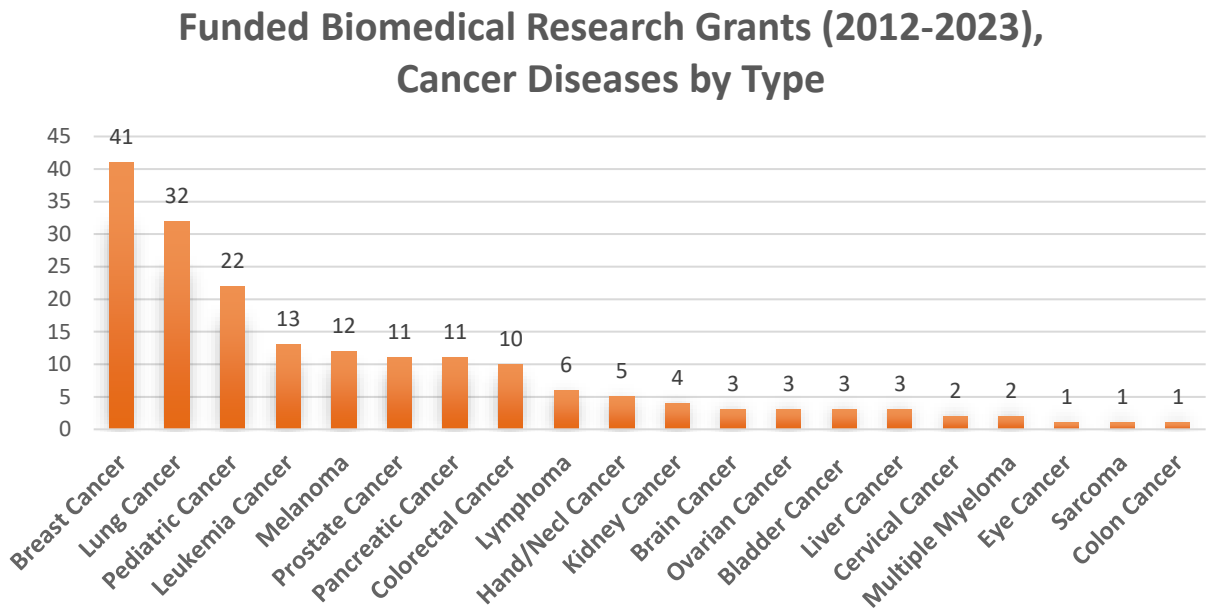


Exhibit 1: Florida Biomedical Research Grants - Cancer by Disease Type; Source: FDOH Biomedical Research Division Records, https://www.floridahealth.gov/provider-and-partner-resources/brac/reports-and-publications.html.

Three Patient Stories that Demonstrate the Impact of Grant-Funded Clinical Trials

Data and statistics on grant awards tell only part of the biomedical research program story. Another component comes from patients who benefit from grant-funded clinical trials. Below are three testimonials from cancer patients to put the importance, significance, and real-world impact of this research into perspective.

“It meant everything just being able to try something to fight this cancer after being told by previous doctors, ‘we don’t know how to treat this,’” said the patient. “Just seeing the doctors that I had at Moffitt really fighting for me and, trying to find something that was a good fit and feeling like they’ve studied it enough to know what might work felt like they were really trying to be a fighter on my side. It was just what I needed at that moment because that’s a devastating diagnosis.”

“The patient says he has finally gotten to the normalcy he has been striving for after participating in a CAR T clinical trial. “The puzzle pieces have come into place. I have a new lease on life. The worry of disease coming back isn’t there anymore,” he said. He also offers this advice to patients who are given the option of CAR T-cell therapy: “Don’t focus on the negatives that could happen with the treatment. Stay positive and focus on how this will get you through to the next chapter of your life.”

“I was excited about the opportunity to participate in a trial. I actually gave up free treatment at the VA to participate. I have a great relationship with my research coordinators, and they make me feel at ease and comfortable to ask questions about my care. They are more available than the doctors or physician assistants, and I appreciate the time they spend with me. They have helped me maintain a positive outlook through my treatment.”

Biomedical Research Advisory Council Membership and Goals.

The Biomedical Research Advisory Council (BRAC) advises the State Surgeon General regarding the direction and scope of the biomedical research program (s. 215.5602(4), Florida Statutes). BRAC's responsibilities 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 BRAC members, as of July 2023, are listed below. There are currently two vacancies. (Biographical statements or curriculum vitae available upon request):

- Daniel Armstrong, PhD (Chair), Professor and Special Senior Advisor to the Chair, Pediatrics, Director, Mailman Center for Child Development, University of Miami Miller School of Medicine Seat: American Cancer Society

- Stephen Black, PhD, Director, Center for Translational Science, Florida International University; Seat: Governor

- Nicole de Lara Puente, Chief Executive Officer, Live Like Bella childhood Cancer Foundation; Seat: Governor

- Shaye Moskowitz, PhD, Neurosciences Medical Director, Broward Health Physicians Group, Seat: Governor

- Akram Shibani, MD, Ascension Medical Group, St. Vincent's Lung Institute; 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

- Roxana S. Dronca, MD, Director, Mayo Clinic Comprehensive Center; Seat: House of Representatives

- Vacant Seats: American Lung Association and American Heart Association

Strategic Goals

The BRAC maintains a strategic plan for Florida's biomedical research funding that defines the objectives to be accomplished in specific timeframes. The strategic plan focuses on the health impact of research and making Florida a destination for cancer care and research. The ten-year cycle for the current strategic plan ends in 2024 and will be updated to align with current statewide cancer priorities. The strategic goals are included in the annual funding opportunity announcement.

Biomedical Research Awards for the 2022-23 Funding Cycle

Awards for the Bankhead-Coley Program, King Program, and Live Like Bella Initiative research grants for FY 2022-23 are presented in Exhibit 2:

Exhibit 2: Biomedical Research Awards by type and program source, FY 2022-23.		
Prevention and Treatment	These awards focus on research related 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.	11 Bankhead-Coley Programs 5 King Programs 11 Live Like Bella Initiatives
Technology Transfer	This grant aims to stimulate technology transfer activities for promising research discoveries that could lead to innovations in the prevention, diagnosis, treatment, and/or cure of cancer and strengthen a project's economic feasibility and commercialization prospects.	3 Bankhead-Coley Programs
Health Disparities	This research contributes to reductions in deaths due to the cancers listed above resulting from health disparities due to race, ethnicity, or income.	1 King Program
Tobacco Use	This research funding aims to reduce tobacco use in children, adolescents, and adults.	1 King Program
Screening	This research focuses on improving screening accuracy and detection for high-risk subgroups, and/or improved implementation of a cancer screening program that results in an increase in early detection or prevention of at least one of the cancers listed above.	1 Bankhead-Coley Program
Treatment-Related Morbidities	This funding 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 King Program 2 Live Like Bella Initiatives

Exhibit 2: Biomedical Research Awards by type and program source, FY 2022-23

The following Exhibits 3, 4, and 5 show the applications submitted and funding awarded for the Bankhead-Coley Program, the Live Like Bella Initiative, and the King Program over time.

Exhibit 3: Bankhead-Coley Program Applications and Funded Projects

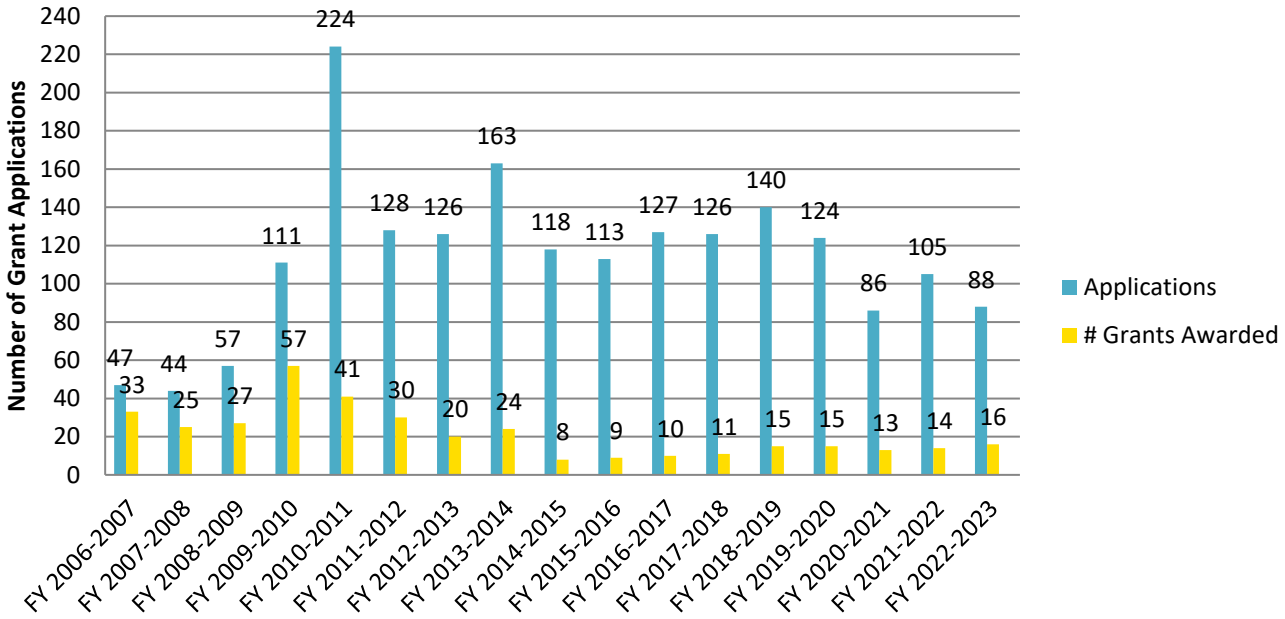


Exhibit 3: Bankhead-Coley Program Applications and Funded Projects; Source: FDOH Biomedical Research Division Records, https://www.floridahealth.gov/provider-and-partner-resources/brac/reports-and-publications.html.

For FY 2022-23, 88 applications were submitted in response to the Bankhead-Coley Funding Opportunity Announcement (FOA) and 16 cancer research projects were awarded.

Exhibit 4: Live Like Bella Applications and Funded Projects

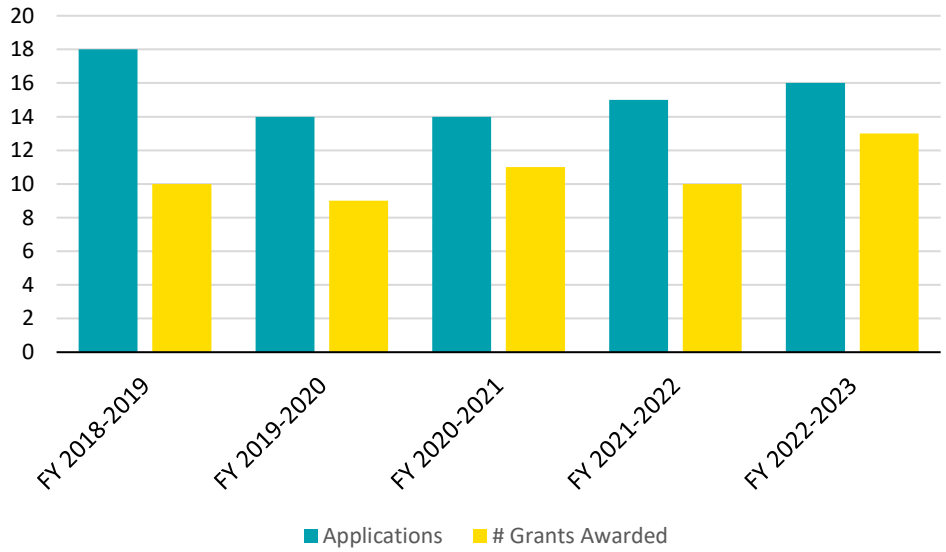


Exhibit 4: Live Like Bella Applications and Funded Projects; Source: FDOH Biomedical Research Division Records, https://www.floridahealth.gov/provider-and-partner-resources/brac/reports-and-publications.html.

For FY 2022-23, 18 grant applications were submitted in response to the Live Like Bella FOA, and 13 pediatric cancer research projects were awarded. As the program continues to become establishes, more grant applications will be submitted.

Exhibit 5: King Program Applications and Funded Projects

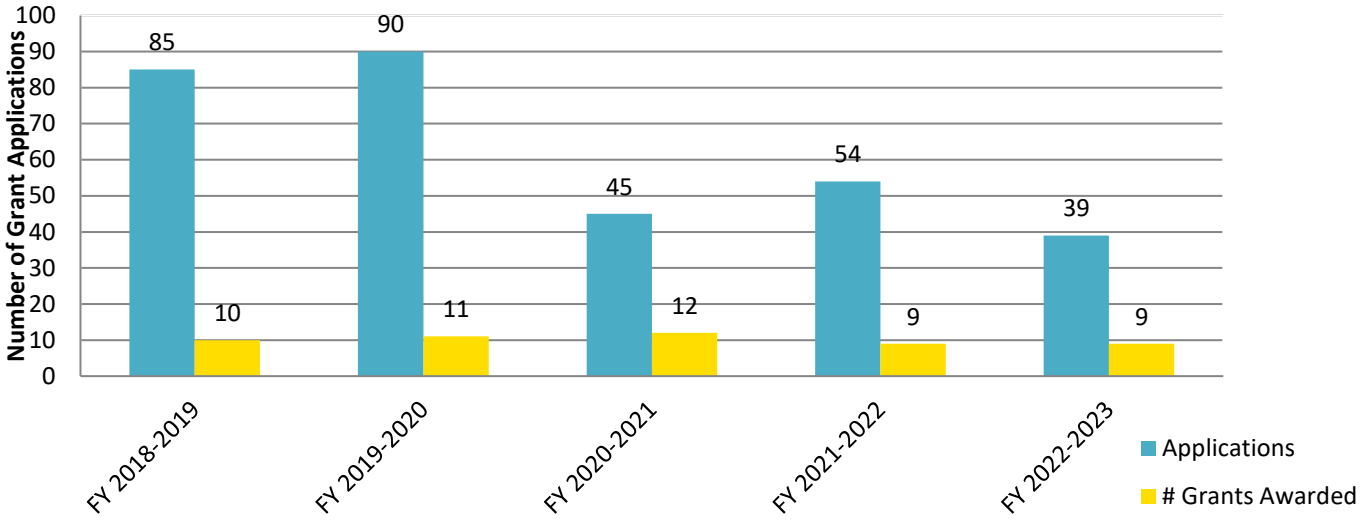


Exhibit 5: King Program Applications and Funded Projects; Source: FDOH Biomedical Research Division Records, https://www.floridahealth.gov/provider-and-partner-resources/brac/reports-and-publications.html.

For FY 2021-22, 39 applications were submitted in response to the King Program FOA, and nine tobacco-related disease research projects were awarded.

Table 1: Awarded Institutions 2006-2023

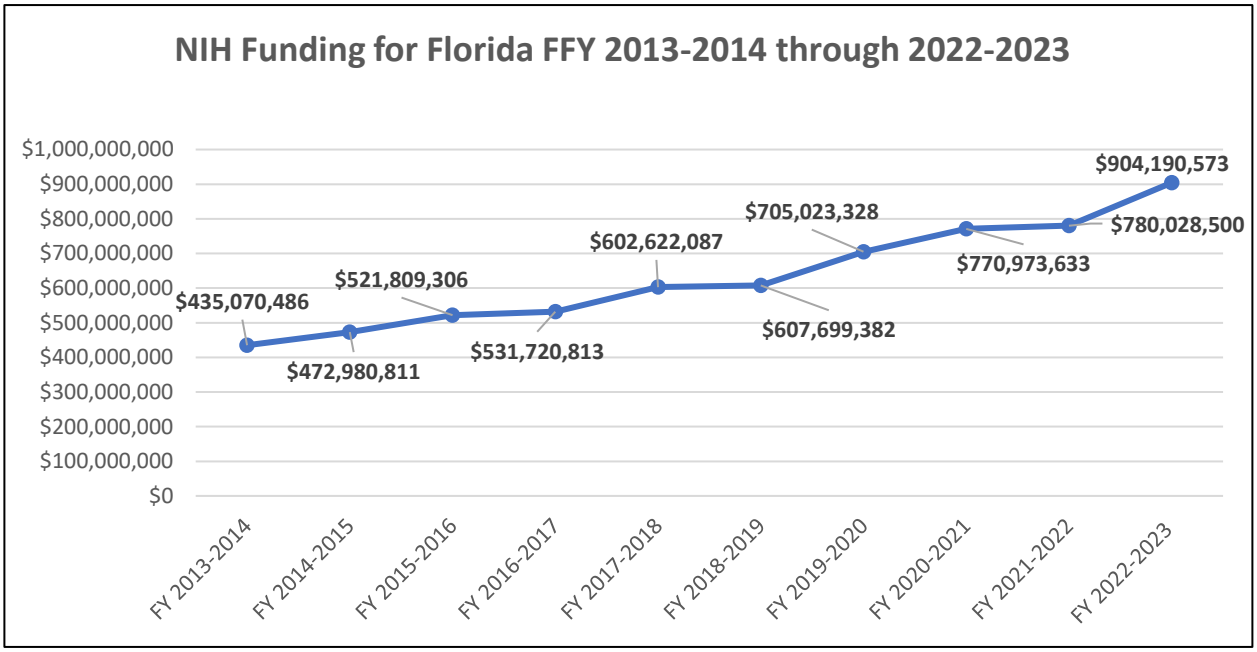
Florida's research infrastructure for cancer and tobacco-related diseases continues to expand. Table 1 includes a list of the institutions and organizations that have received research funding to create and build the research infrastructure.

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

In FFY 2022-23, the state saw an increase in total NIH funding from $780 million to over $904 million (see Table 1). However, Florida remains 12th in the United States for total NIH biomedical funding awarded (See Table 2). These results reflect Florida's initiative to expand upon research to improve scientific understanding of various diseases and health disparities.

Table 2: NIH Research Funding Received by Florida Researchers Continues to Increase



Source: NIH Research Portfolio Online Reporting Tools (RePORT) Data as of 10/04/2023. "Data will include R&D contracts, fellowships, other grant awards not yet past their budget start date." www.report.nih.gov/award/index.cfm.

Table 3: Top 20 Recipients for NIH Research FFY 2022-2023

National Institutes of Health Biomedical Research State Funding and Rankings FFY 2022–2023			
State	**# of Awards**	**Total Funding**	**Rank**
California	8925	$5,224,061,739	1
New York	6409	$3,479,293,380	2
Massachusetts	5842	$3,417,990,511	3
Pennsylvania	4175	$2,184,387,344	4
North Carolina	2740	$2,089,470,303	5
Texas	3672	$1,815,431,719	6
Maryland	2343	$1,401,232,773	7
Washington	1757	$1,221,769,407	8
Illinois	2327	$1,199,865,404	9
Michigan	1979	$984,458,682	10
Ohio	2009	$981,891,628	11
Florida	**1688**	**$904,190,573**	**12**
Missouri	1548	$788,381,289	13
Connecticut	1490	$765,815,881	14
Tennessee	1342	$743,889,264	15
Georgia	1489	$743,071,990	16
Minnesota	1294	$710,742,414	17
Wisconsin	1044	$646,485,715	18
Virginia	1007	$561,782,074	19
Colorado	1254	$539,742,498	20

(*Source: NIH Research Portfolio Online Reporting Tools (RePORT). "Data as of 10/04/2023. Data will include R&D contracts, fellowships, other grant awards not yet past their budget start date." www.report.nih.gov/award/index.cfm.*

Biomedical Research Grant Funding Long-Term Impact Survey Results, 2023

Overview and Methodology

Outcomes associated with cancer research programs may be difficult to identify in the short-term. As a result, Department staff, on behalf of the BRAC, surveyed Biomedical research grant awardees for the 2011-2012 and 2012-2013 funding cycles to assess long-term accomplishments. Staff identified 78 distinct Principal Investigators (PIs) and conducted further research to determine if the PIs were still in-state and able to be contacted for responses. Of the 78, about 60% (N=48) were still at universities within the state with valid email addresses. The remaining PIs were retired, deceased, not locatable, in the private sector without contact information or had left the state. A short 10-question survey was sent via email.

Results Summary

Overall, the grant recipients felt the funding had a positive long-term impact on their careers. The majority (54.55%) received the funding early in their careers and reported that this funding helped them receive tenure or promotion (72.73%). Likewise, 72.73% felt the grant funding had a high impact on their research program long term. When responding to open-ended questions about how the biomedical research funding had helped their careers, respondents indicated that funding led to development of new cancer treatments, patents, and drugs, provided seed and/or gap funding, research program expansion, receiving federal funding and multiple publications (more detail provided below). Approximately half of respondents (54.55%) stated the biomedical grant funding provided the basis for research patents and/or treatments currently used in patient care. A total of 90% responded the funding led to the recipient receiving other federal grant funding, e.g., NIH, Department of Defense and Veteran's Administration. 45% stated research staff and/or post-doctoral researchers who worked with the PIs on their projects went on develop their own research programs.

Respondents were asked to share success stories from their biomedical grant experiences. Highlights from respondent experiences included the following:

- Biomed Research grant provided significant financial support for research projects that were not funded by federal agencies such as the National Cancer Institute (NCI) and NIH. Supported by this program, some of the early-stage research projects developed into more substantial investigations, which established the ground for the application of findings for potential clinical development.
- Early work in minimally invasive staging of lung cancer has now transformed the field from surgical (mediastinoscopy) to endoscopic (endobronchial fine-needle aspiration (FNA)), as well as led to related advances in early pancreas cancer detection.
- This funding resulted in a novel cancer therapy that is currently in clinical trials, with the technology acquired by a multi-national Pharma company. The project allowed development of Natural Killer (NK) cell expansion technology that was patented and formed the core of a spin-off company CytoSen Therapeutics. CytoSen with the NK cell technology has essentially been acquired twice. CytoSen was acquired first in 2019 by Dutch company Kiadis. The NK cell expansion technology became the core business for Kiadis and later resulted in acquisition by French pharma company, Sanofi. The

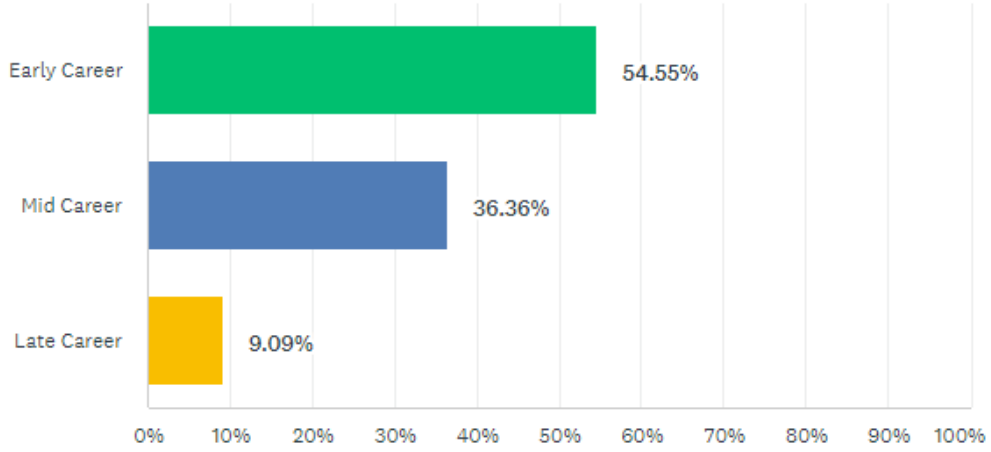
technology that started off at the University of Central Florida (UCF) with the Florida Department of Health (FL DOH) funding is now further being researched within Sanofi in Europe and the United States and is now conducting multiple clinical trials of the NK cells for treatment of leukemia.

- The technician working on a research grant project went to medical school and is now a resident of radiology. His father, a citizen of Palm Beach, came and told the Principal Investigator that research had transformed his son's life.

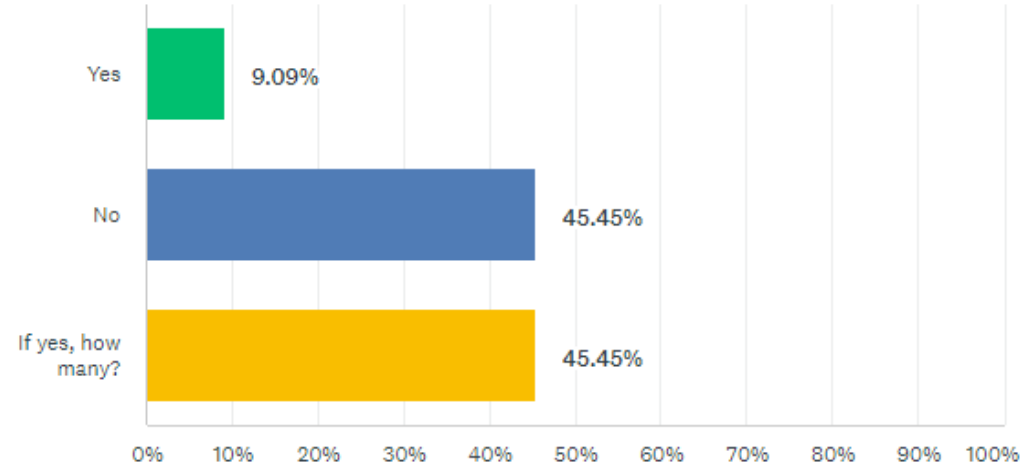
Additional successes are included in individual project updates included in this report.

Long-Term Impact Survey Questions and Results:

Question 1: At what point in your career did you receive the 2011/2012 and/or 2012/2013 Florida Department of Health Biomedical Grant funding?

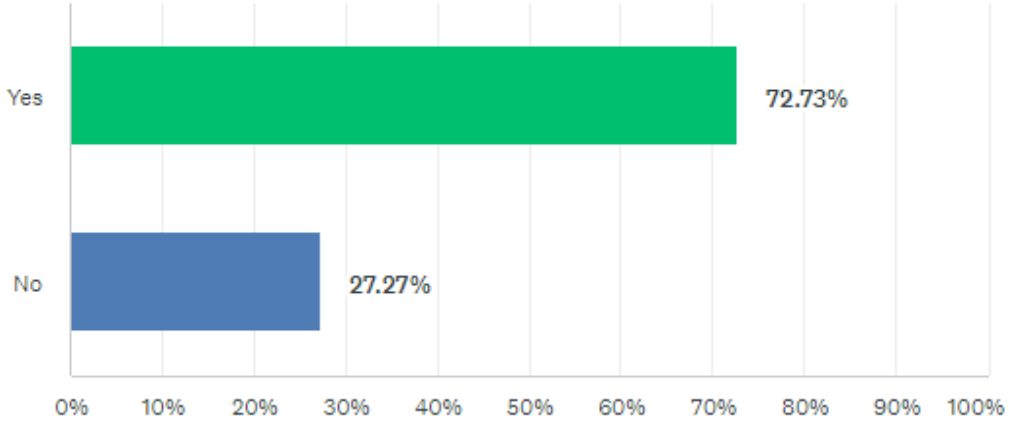


Question 2: Since you received the 2011/2012 and/or 2012/2013 grant funding, have you received any other Florida Department of Health Biomedical Research Grants?

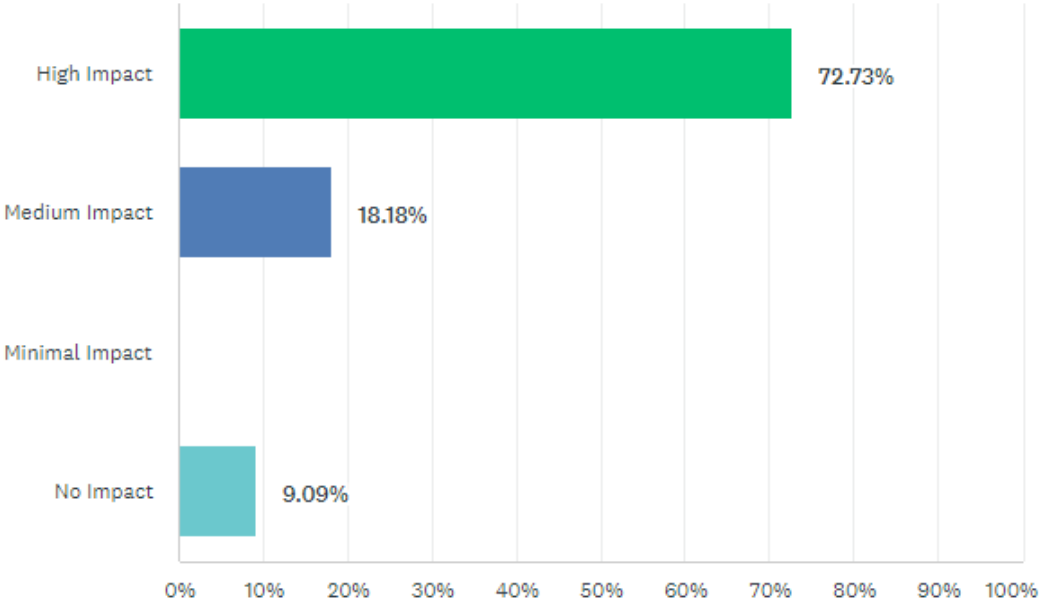


Five respondents indicated they had received at total of seven additional Department Biomedical Research Grants.

Question 3: Did the Biomedical Research Grant funding help support you earn tenure or other promotion?



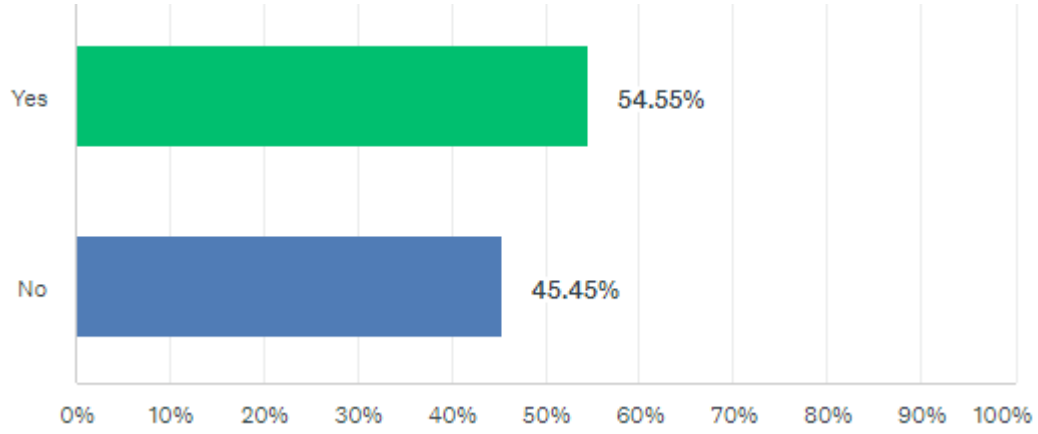
Question 4: How much impact did this research grant funding have on your research program long-term?



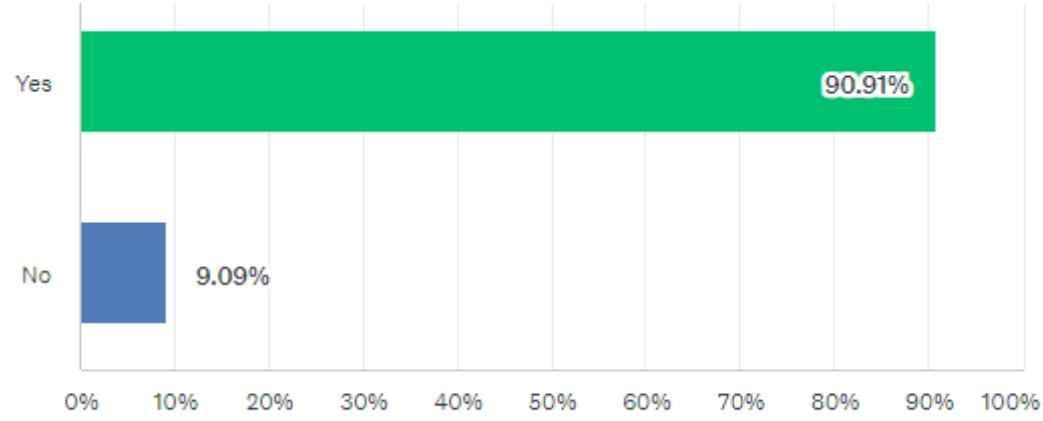
Question 5: Briefly describe how the Biomedical Research Grant Program helped your research career. Answers to this open-ended question are included below.

- Allowed continuation of research during loss of NIH funding.
- Tested new anticancer drug which was patented and distributed to other researchers.
- Help[ed] me to establish a research program in Epigenetics.
- Florida Biomedical Research Grant Program funding enabled me to test an idea that blood vessels are a sanctuary site for cancer, which led to [the development of] new cancer treatments that I translated into clinical treatments for cancer patients.
- The New Investigator Research grant enabled me to develop and expand my research program, which has led indirectly to an NIH R01 grant and directly to an excellent impact score (4 percentile) within the NCI payline (funding decision is pending council meeting).
- By providing significant financial support for laboratory research that was not funded by federal agencies such as NCI/NIH.
- FL DOH grant helped me to establish my laboratory and my scientific career.
- This was my first major extramural grant in Florida. The seed funds led to future NIH R01 and other funding and promotion to Professor and eventually endowed Professor of Medicine at Mayo.
- It is facilitating the collection of data that will support a new research program.
- The funding by FDOH led to multiple publications, patents, and additional funding from NIH. Several compounds discovered as a result of FDOH funding are in pre-clinical trials showing promise for further development.

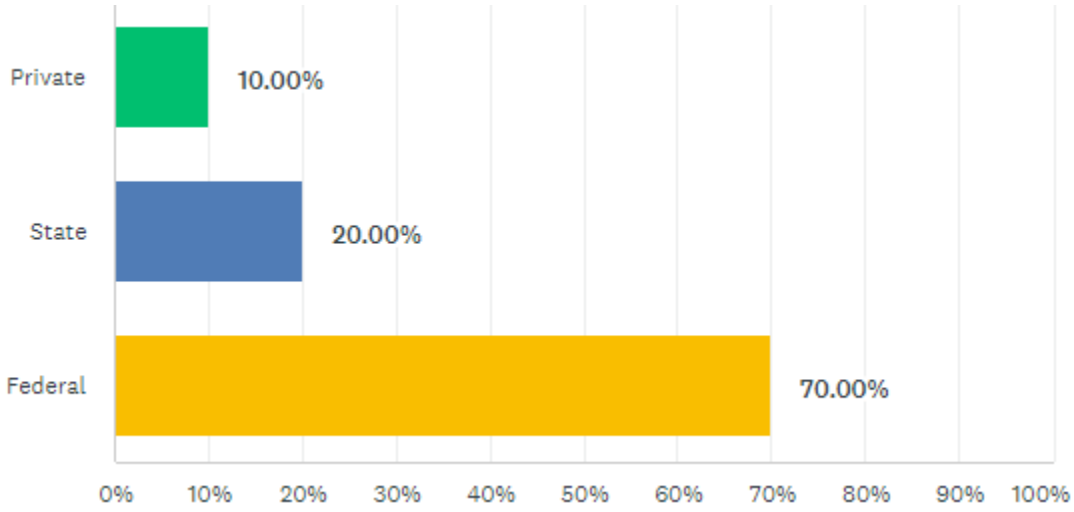
Question 6: Did this Biomedical Grant funding provide the basis for any research patents and/or treatments currently being used in patient care?



Question 7: Did the Biomedical Grant funding subsequently help you receive other grant funding?



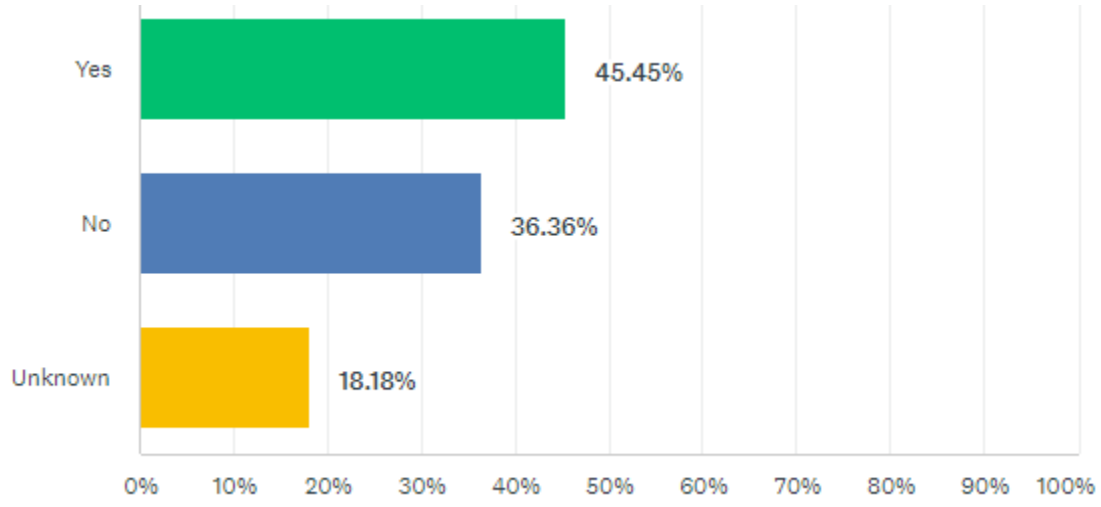
Question 8: If Yes to Question 7, what was the source of the other grant funding?



Respondents further identified the various types of federal funding they received:

- One NIH R01
- NIH, DOD
- NIH R01
- R01 NIH DoD
- NIH
- NIH and VA
- NIH

Question 9: Did any research staff or post-doctoral researchers who worked on the Biomedical Research grant go on to develop their own research programs?



Question 10: Please share any success stories that resulted from receiving the Biomedical Research grant funding. These could include stories about researchers, research staff and/or research participants. Answers to this open-ended question are listed below.

- Publication of research continued due to this grant.
- My graduate student at that time went on to develop successful career in bioinformatics and now is a faculty at the University of Southern California.
- The technician working on the project went to medical school and is now a resident of radiology. His father, a citizen of Palm Beach, came to my office and told me that research had transformed his son's life.
- This state funding kept me in the State of Florida and enabled me to invent new treatments for cancer patients in Florida. Florida is gravely underfunding this program compared to Texas and other large states that are serious about investing in their research workforce. The Biomedical Medical Research Program needs five times more funding.
- The New Investigator Research grant really helped me develop and expand my research program, get promoted, and obtain federal funding. I am now serving as an ad hoc reviewer for an NIH study section.
- Biomed Research grant provided significant financial support for research projects that were not funded by federal agencies such as NCI/NIH. Supported by this program, some of our early-stage research projects developed into more substantial investigations, which established the ground for the application of our findings for potential clinical development.
- This funding resulted in a novel cancer therapy that is currently in clinical trials, with the technology acquired by a multi-national Pharma company. The project allowed development of NK cell expansion technology that was patented and formed the core of

a spin-off company CytoSen Therapeutics. CytoSen with the NK cell technology has essentially been acquired twice. CytoSen was acquired first in 2019 by Dutch company Kiadis. The NK cell expansion technology became the core business for Kiadis and later resulted in acquisition by French pharma company, Sanofi. The technology that started off at UCF with FL DOH funding is now further being researched within Sanofi in Europe and the US and is now conducting multiple clinical trials of the NK cells for treatment of leukemia.

- Our early work in minimally invasive staging of lung cancer has now transformed the field from surgical (mediastinoscopy) to endoscopic (endobronchial FNA), as well as led to related advances in early pancreas cancer detection.
- An MD/PHD student from underrepresented background generated data to support presentation in the national and international meetings.
- Dr. Minond, the PI of the Esther and James King-funded grant, discovered novel anti-melanoma compounds that are currently showing promise in pre-clinical trials.

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

Fiscal Year 2022-2023 Newly Awarded Active Grants

Funded Fiscal Year 2022-2023

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
23B01	University of Florida	Lizi Wu, PhD	$587,097.00	3/31/26	No	No	No
23B02	University of Florida	Chengguo Xing, PhD	$294,300.00	3/31/25	No	No	No
23B03	University of Florida	Brian K. Law, PhD	$100,000.00	9/30/23	Yes	Yes	No
23B04	University of Florida	Samsun Lampotang, PhD, FSSH, FAIMBE	$100,000.00	9/30/23	No	No	No
23B05	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Joseph Markowitz, MD, PhD	$588,600.00	3/31/26	No	No	No
23B06	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Paulo Rodriguez, PhD	$100,000.00	9/30/23	No	No	No
23B07	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Michael Jain, MD, PhD	$1,471,500.00	9/30/27	No	No	No
23B08	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Jose Guevara-Pantino, MD, PhD	$294,300.00	3/31/26	No	No	No
23B09	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Ahmad Tarhini, MD, PhD	$535,908.00	3/31/26	No	No	No
23B10	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Lixin Wan, PhD	$588,600.00	3/31/26	No	No	No
23B11	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Eric Lau, PhD	$294,600.00	5/31/26	No	No	No
23B12	Florida Atlantic University	Esther Guzman, PhD	$588,600.00	3/31/26	No	No	No
23B13	University of Central Florida	Deborah A. Altomare, PhD	$588,600.00	3/32/26	No	No	No
23B14	University of Central Florida	Otto Phanstiel, PhD	$588,600.00	3/31/26	No	No	No
23B15	University of Miami	Barbara Bedogni, PhD	$588,600.00	3/31/26	No	No	No
23B16	University of Miami	Stephan C. Schurer, PhD	$1,471,500.00	3/31/26	No	No	No

1. **Grant#:** 23B01 Elucidating and Targeting INSL4 Signaling in Lung Cancer

Principal Investigator: Lizi Wu, PhD

Organization: University of Florida

Summary: Lung cancer is the leading cause of cancer deaths in the United States and worldwide. Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases, and 15-30% of them carry inactivating genetic alterations in the tumor suppressor gene liver kinase B1 (LKB1), also known as Serine/threonine kinase 11 (STK11). Lung cancer with LKB1 inactivation is very aggressive and treatment-refractory with no available targeted therapies. Because it is difficult to target inactive or absent tumor suppressors directly, exploring the effector targets or pathways influenced by LKB1 inactivation could provide viable therapeutic targets. To understand the critical signaling downstream of LKB1 inactivation, the research team performed a global transcriptome profiling study and identified INSL4 (insulin-like 4) as a new LKB1-regulated target gene. INSL4 is a secreted protein that belongs to the insulin superfamily. INSL4 is restrictively expressed in the placenta and has no undetectable expression in normal adult tissues; yet its expression is aberrantly upregulated in human LKB1-mutant NSCLC cell lines and primary tumors. Importantly, researchers discovered that LKB1-mutant NSCLCs depend on INSL4 signaling for growth and survival, thus identifying a novel vulnerability of LKB1-mutant lung cancer. Based on these lines of evidence, the researchers hypothesize that INSL4 signaling represents an important and safe therapeutic target in LKB1-mutant lung cancer. Therefore, the objectives of this research are to gain a better mechanistic understanding of INSL4 tumor-promoting functions and to evaluate INSL4-targeting strategies in blocking lung

cancer in preclinical models. This research is anticipated to provide new mechanistic insights into aberrant INSL4 signaling in lung cancer progression and guide the future development of targeted therapy for this prevalent aggressive LKB1-mutant 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.

2. **Grant#:** 23B02 AB-free kava in lunch cancer chemoprevention

Principal Investigator: Chengguo Xing, PhD

Organization: University of Florida

Summary: The goal of this grant is to evaluate the potential of AB-free kava in reducing lung cancer risks via a tobacco smoke-induced lung carcinogenesis animal model. During the past funding period, which has only three months, the research team has successfully started the projects with the following activities: Supplies and replacement parts for the smoking machine have been ordered and received. The smoking machine has been set up and tested with conditions optimized. A pilot group of mice (n=10) have been ordered, received, and tested for both smoking exposure level (serum cotinine quantified to be around two micrometers (uM), comparable to the level of plasma cotinine among heavy smokers), the use of metabolic cage, and powdered diet has been established. Various biological samples have been collected from this pilot study. Several endpoints are currently under investigation, such as inflammation markers and tobacco exposure, tobacco ingredient metabolism, and stress marker changes. An Institutional Animal Care and Use Committee (IACUC) amendment has been made to include non-invasive lung function measurement, which has been approved with the corresponding document submitted to the Florida Department of Health on June 14, 2023. The research team has also ordered a second round of mice, additional supplies for metabolic cages, and additional cigarettes have been ordered. In summary, adequate progress has been made to this grant during the past three-month funding period. The main goal of the next funding period is to further optimize the experimental condition and perform the proposed long-term study. An interim analysis of the pilot study will take place as well during 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.

3. **Grant#:** 23B03 Optimizing novel small molecule activators of Death Receptor 5 for breast cancer therapy

Principal Investigator: Brian K. Law, PhD

Organization: University of Florida

Summary: The multidisciplinary team has developed a new class of anti-cancer agents termed Disulfide bond Disrupting Agents (DDAs). DDAs act by inhibiting a novel subset of Protein Disulfide Isomerases (PDIs), anterior gradient protein 2 homolog (AGR2), protein disulfide isomerase A1 (PDIA1), and endoplasmic reticulum-resident protein 44 (ERp44). DDA-mediated inhibition of AGR2, PDIA1, and ERp44 causes changes in the disulfide bonding, stability and signaling functions of key disulfide bond-rich receptors that control the life or death of cancer cells. Importantly, DDAs robustly induce apoptosis of human breast tumor cells in animal models without observable effects on adjacent normal tissues. Since these initial studies, new generations of more selective and potent DDAs have been generated. However, the in vivo pharmacological properties of these new compounds, and their engagement of AGR2, PDIA1, and ERp44 and consequent DR5 upregulation and activation in intact tumors, have not been investigated. The results of these studies will focus on follow-up IND enabling efforts on the DDA candidate with the greatest potential for success in clinical trials. The Specific Aims of the proposed project are to increase the commercial value and translational potential for advancing Disulfide bond Disrupting Agents (DDAs) as a novel therapy for breast cancer by evaluating the stability and metabolism of the most promising DDAs, examining potential markers of DDA toxicity to normal cells and tissues, and demonstrating DDA engagement of its target proteins, AGR2,PDIA1, and ERp44 in tumors in vivo. Further, DDA effects on the levels and signaling functions of HER1-3, and Death Receptors 4 and 5 (DR4/DR5), in tumors and normal tissues will be assessed. To date, these studies showed that dFtcyDTDO is highly stable against human liver and intestinal enzymes and did not show measurable metabolism in the one-hour treatment period. Thus, dFtcyDTDO may be suitable for advancement to clinical trials against cancer. Upcoming studies include examining if dFtcyDTDO induces the same biochemical effects on tumors in vivo as in cancer cells in culture. Further, markers of tumor sensitivity to DDA treatment will be evaluated, including over expression of the MYC and Epidermal Growth Factor Receptor (EGFR) oncoproteins.

Follow on Funding: Tirosh, B, PI; Mechanisms of adaptation to chronic ER stress by the PDI network. NIH R01 12/01/2023-11/30/2028. Pending.

Collaborations: Ronald K. Castellano, PhD, Professor, University of Florida (UF) Department (Dept.) of Chemistry: Dr. Castellano's laboratory synthesizes the DDA anticancer compounds under study in the project and has produced new DDAs since the project began that are currently being evaluated.

Coy D. Heldermon, MD, PhD, Associate Professor, UF Dept. of Medicine: Dr. Heldermon collaborates on the animal studies and together with the Law laboratory are evaluating DDA anticancer activity in vivo and are developing new models of Triple-Negative Breast Cancers (TNBCs), with the goal of using DDAs against TNBCs in African American patients to reduce the breast cancer survival disparity experienced by African American women.

Roberto Sitia, PhD, Professor, Managing Director, Università Vita-Salute San Raffaele, Italy: One of the DDA target proteins is the disulfide isomerase ERp44. In fact, DDAs are the only reported inhibitor of ERp44. Dr. Sitia is a world expert on the role of ERp44 in protein folding and has requested some DDA compound for studying the effects of pharmacological inhibition on the cycling of ERp44 between the endoplasmic reticulum and Golgi compartments within the secretory pathway.

Boaz Tirosh, PhD, Professor, Dept. of Biochemistry, Case Western Reserve University, Cleveland Ohio: Dr. Tirosh discovered the process of "selective Endoplasmic Reticulum retention" (sERr) in which proteins become arrested in the ER in high-molecular mass disulfide-bonded complexes orchestrated by ERp44. In collaboration with Dr. Tirosh, the researchers are determining how DDAs alter sERr by inhibiting ERp44. This collaboration has already involved exchanges of research materials and experimental results.

Journals: None at the time of reporting.

Patents: Law, BK, Castellano, R, Ferreira, R, inventors; NOVEL SMALL MOLECULE ANTICANCER AGENTS. US patent 10,813,904. October 27, 2020.
Patent Applications Currently Pending

Law, BK, Jahn, S, et al, inventors; University of Florida Research Foundation, assignee. NOVEL SMALL MOLECULE ANTICANCER AGENTS, COMBINATIONS AND USES THEREOF. US National Phase Application No.: 17/912,477. September 16, 2022, International Patent Application No.: PCT/US2021/022542. Date: March 16, 2021; Publication Date: April. 20, 2023

Law, BK, Castellano, R, inventors; University of Florida Research Foundation, assignee. INHIBITION OF THE PDI FAMILY MEMBERS AGR2, PDIA1, AND ERP44 FOR THERAPEUTIC TREATMENT AND USE IN PREDICTIVE DIAGNOSTICS/MONITORING FOR TREATMENT REGIMENS. Application Nos.: PCT/US2022/011961 - WGS Ref. No.: U1195.70190WO00; International Filing Date: January 11, 2022, Rec. Date: March 15, 2022.

Law, BK, Castellano, R, inventors; University of Florida Research Foundation, assignee. ANTICANCER COMPOUNDS AND USES THEREOF. Application No.: US 63/135,979; Filing Date: January 11, 2022.

Law, BK, Jahn, S, et al, inventors; University of Florida Research Foundation, assignee. NOVEL SMALL MOLECULE ANTICANCER AGENTS, COMBINATIONS AND USES THEREOF. Application No.: US 62/990,544; Filing Date: March 17, 2020.

Law, BK, Castellano, R, inventors; University of Florida Research Foundation, assignee. NOVEL SMALL MOLECULE ANTICANCER AGENTS, COMBINATIONS AND USES THEREOF. Application No.: U1195, 70134US01; Filing Date: June 15, 2018.

4. **Grant#:** 23B04 3D perspective visualization for increasing prostate biopsy accuracy

Principal Investigator: Samsun Lampotang, PhD, FSSH, FAIMBE

Organization: University of Florida

Summary: This proposal was to build and retrofit an electromagnetic (EM) tracking guidance system to a micro-ultrasound (29 MHz) transrectal probe used for prostate biopsy and validate the proposed system by evaluating its accuracy in placing biopsy cores at the intended locations in the prostate. The research team also aims to obtain Abbreviated Investigational Device Exemption from the University of Florida (UF) Institutional Review Board (IRB) 01 and conduct a first in human use of the system in 20 consenting patients scheduled for prostate biopsy at the UF Health Urology Clinic. The proposed system visualized prostate biopsy (vPBx) offers a three-dimensional (3D) perspective visualization planning, guidance, and feedback system for

systematic and/or targeted prostate biopsy via the transrectal or transperineal approach. By reducing prostate biopsy false negatives via increased accuracy, the proposed system addresses these Bankhead-Coley Research Priorities: “Improve screening accuracy and detection in high-risk groups,” and Technology Transfer Feasibility (TTF). Currently, freehand TRUS-guided systematic prostate biopsy (sPBx) is predominantly used for initial biopsy but produces a high percentage of false negatives (21-47%). The requirement for an MRI scan and radiologist fees remains a significant hurdle that keeps free PBx out of reach of patients, especially those suffering from health disparity. An accessible, accurate PBx system that does not need a prior MRI scan is needed that is easy to use for both systematic and targeted biopsy, via the transrectal or transperineal route. The researchers propose to build such a system with the requested bridge funding. Specifically, the research team aims to build and verify the hardware and software for retrofitting a visualized prostate biopsy system to an ExactVu (EV29L) micro-ultrasound probe to provide planning, guidance, and feedback during systematic and targeted transperineal prostate biopsy. The next step in the grant is to develop and verify the software for implementing and interfacing of the vPBx to actual prostate biopsy equipment in the UF Medical Plaza.

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#:** 23B05 Establishing the role of aberrant splice variants as a clinical biomarker in clear cell renal cell carcinoma

Principal Investigator: Joseph Markowitz, MD, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: In 2022, 99,780 cases of melanoma with 7,650 deaths are predicted in the United States. Florida shares a major burden of this disease with 9,650 cases estimated in the state. In the past few years, melanoma therapy has undergone a revolution with therapies that target the immune system. However, even with these advancements in immune based therapies upwards of 60% of people treated with single agent anti-PD-1 people will not respond. Even with combination anti-PD-1/CTLA-4 or anti-PD-1/LAG3, response rates only increase to 50 to 60% at the cost of significantly increased toxicity. Thus, new strategies are needed to improve therapeutic outcome. This project is led by a physician scientist, Joseph Markowitz, MD, PhD, who investigates novel mechanisms that may be exploited to treat melanoma. IFx-Hu2.0 is a therapeutic agent injected into tumors that expresses part of a bacterial protein to stimulate immune recognition of the tumor and kill melanoma cells. IFx-Hu2.0 has been studied in murine and equine melanoma and it stimulates an immune response to control the cancer. Based on the preclinical work and the first-in-human trial researchers hypothesize that IFx-Hu2.0 primes the immune response for effective immunotherapy. The specific aims of this study are: 1) utilize results from clinical trial samples to guide murine experiments to investigate the systemic immune response elicited by IFx-Hu2.0; 2) study how IFx-Hu2.0 alters the tumor microenvironment to sustain the immune response; 3) study how IFx-Hu2.0 facilitates response to anti-PD-1 and other immune therapies in its class of therapeutics (checkpoint blockade). By

pursuing this research, research staff will gain valuable insight into the possibility of adding IFx-Hu2.0 to checkpoint blockade therapy (anti-PD-1 or combination anti-PD-1/CTLA-4, anti-PD-1/LAG3) in future clinical trials and the mechanisms of resistance to checkpoint blockade therapy 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.

6. **Grant#:** 23B06 Mitochondrial stress-related proteins regulate myeloid subsets in lung and melanoma tumors

Principal Investigator: Paulo Rodriguez, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: The immunosuppressive tumor microenvironment (TME) orchestrated by advanced malignancies represents a key driver for tumor progression and a challenge for the successful development of curative immunotherapies. To date, however, the approaches to clinically block the regulatory effects of myeloid cells in tumors are limited to myelosuppressive agents or inhibitors that are only partially effective. Thus, alternative strategies to overcome the immunosuppressive and pro-tumorigenic actions of myeloid cells in cancer are expected to have a significant clinical impact. Infiltration of myeloid cells into tumors makes them exposed to stress conditions, including hypoxia, nutrient deprivation, and elevated reactive oxygen and nitrogen species, which trigger overactivation of endoplasmic reticulum (ER) stress. Recent manuscripts demonstrated that maladaptive signaling by major arms of the ER stress responses, protein kinase RNA-like endoplasmic reticulum kinase (PERK) and C/EBP homologous protein (CHOP), impaired anti-cancer immunity by intrinsically altering the metabolic and functional activity of myeloid cells in tumor beds. Thus, researchers hypothesize that the chronic overactivation of PERK→CHOP provokes detrimental UPRmt in tumor-linked myeloid cells, which results in a program that allows mitochondrial adaptation to stress and sustains immune evasion. Researchers propose the following Specific Aims: Determine the role of LonP1 in different myeloid subsets in tumor beds and characterize the LonP1 signalosome in tumor-linked myeloid 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.

7. **Grant#:** 23B07 A Clinical Trial of Pirtobrutinib and Brexucabtagene Autoleucel in Patients with Relapsed or Refractory Mantle Cell Lymphoma

Principal Investigator: Michael Jain, MD, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: This project is to conduct a multicenter clinical trial that combines two of the most effective known therapies for patients with relapsed or refractory Mantle Cell Lymphoma (R/R MCL). These are pirtobrutinib, a novel non-covalent Bruton's Tyrosine Kinase inhibitor (BTKi), and brexucabtagene autoleucel, a cluster of differentiation 19 (CD19)-directed chimeric antigen receptor (CAR) T-cell therapy. The primary endpoint is to improve the progression-free survival of patients, meaning that the aim is to increase the number of patients who are free from lymphoma and alive after these therapies. The Bankhead-Coley Award covers conduct of the clinical trial and key correlative science conducted at the Florida sites- the University of Miami and Moffitt Cancer Center and Research Institute, Inc. (Moffitt). The industry partner, Loxo@Lilly is providing pirtobrutinib free of charge for the trial, and funding for conduct of the trial at Stanford Cancer Center in California. Having three sites will allow the trial to meet enrollment quickly in this relatively rare disease. The progress of the project is as follows: The design of the trial is finalized and the primary endpoints with the statistical hypothesis testing parameters are complete. The industry partner Loxo@Lilly has confirmed support and partnership and Stanford is confirmed as a third center for patient enrollment. The trial protocol is nearing final approval. The laboratory manual that harmonizes correlative sample collections between the three trial sites is complete. Internally, arrangements have been made to use a shared IRB and logistics around funding and budgeting have been discussed. The first step is submission to the Scientific Review Committee for comments, followed by submission to the US FDA (Food and Drug Administration) for an Investigational New Drug (IND) application. Following FDA approval, the study will be submitted to the shared Insitutional Review Board (IRB). Once approved by the IRB, final contracting, site initiation visits, and implementation meetings will occur, so that the study may begin enrollment of patients. Study startup at the University of Miami and Stanford sites will occur concurrent to Moffitt, sequenced so that protocol amendments requested by scientific review committees at one site minimize delays in opening at other sites. The shared IRB will allow harmonization of IRB review across the three sites.

Follow on Funding: None at the time of reporting.

Collaborations: As noted above, the project is a multicenter clinical trial. The participating institutions are: Moffitt Cancer Center and Research Institute, Inc. (Overall Pl: Dr. Michael D. Jain), University of Miami Sylvester Comprehensive Cancer Center (Co-Pl: Jay Y. Spiegel), Stanford Cancer Center (Site Pl: Dr. Saurabh Dahiya).

Journals: None at the time of reporting.

Patents: None at the time of reporting.

8. **Grant#:** 23B08 CD3z-Independent Signaling Module for CAR-T Cell Therapies Against Solid Cancer

Principal Investigator: Jose Guevara-Pantino, MD, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: Chimeric antigen receptor (CAR) has emerged as an applicable form of therapy against cancer. CAR consists of an extracellular domain (scFv) that is responsible for binding to the tumor, a transmembrane domain that serves as the anchor to the T cell membrane (TM), and an intracellular (IC) domain responsible for initiating the signaling cascade, cluster of differentiation 3 zeta (CD3 ζ)- chain in the first CAR generation. Given the limited signaling response in the first generation, second-generation CAR included additional domains such as CD28, 4-1BB, or OX-40 to improve responses. Importantly, second generation CAR are effective against liquid malignancies but ineffective against solid tumors. Thus, there is a clear need for the improvement of CAR designs. Natural killer group 2 member D (NKG2D) is a potent co-stimulator of TCR-signaling. However, in celiac disease, an autoimmune disease elicited by gluten intolerance characterized by the destruction of the small intestine, upregulation of NKG2D enables CD8 T cells to recurrently kill in a TCR-independent manner through NKG2D. The Lab has worked on harnessing this property to induce effective anti-tumor T cell responses. The research staff propose that NKG2D can be exploited to improve current CAR-T cell therapies. The research team has generated a series of chimeric antigen receptors (CARs) that recognize CD19 yet lack CD3z. These are central to this study as these receptors aid in the development of improved forms of immunotherapy against cancer. The bypassing of CD3z is crucial, as although it was previously believed to be necessary for the effective function of CART cell therapies, it has also been associated with limiting factors within the therapy. Further studies led to the conclusion that NK receptors can be used as signaling domains in CAR T cells, eliminating the need for the CD3z chain activating domain. Researchers have observed that human T cells expressing an anti-TRP1 (melanoma) CAR are effective at killing melanoma cells in vitro. Next steps involve expressing the TA99 scFv (anti-TRP1) CAR in the context of the NKG2D signaling CAR. Researchers plan to evaluate and compare it with the classical second-generation CAR expressed in 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.

9. **Grant#:** 23B09 Inherited genetic variation as a predictor of the risk of immune related adverse events and the likelihood of clinical benefit

Principal Investigator: Ahmad Tarhini, MD, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: Lung cancer is the number one cause of cancer death in the US. Non-small cell lung cancer (NSCLC) accounts for the majority (80%) of lung cancer diagnoses. NSCLC is divided into two major sub-types, lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). New therapeutic agents targeting major oncogenic drivers of LUAD have led to improved response rates and patient survival. However, due to a lack of well-characterized, validated, and therapeutically actionable oncogenic drivers, similar therapeutic advances for LUSC have not been forthcoming. The research team has identified protein kinase C iota (PRKCi) as an oncogene that drives LUSC tumorigenesis, characterized critical PKCi-dependent oncogenic signaling mechanisms that drive LUSC tumor growth, and identified and

characterized a PKCi inhibitor, Auranofin (ANF), that shows clinical promise for treatment of LUSC. More recently, the research team discovered that PKCi creates and maintains a highly immune suppressive tumor microenvironment (TME) that confers resistance to a-PD-1 in a genetically engineered mouse model (GEMM) of LUAD. The research team recently developed the first GEMM of PKCi-driven LUSC with which to directly assess the role of PKCi in LUSC immunity. Based on the published and preliminary data, the researchers hypothesize that: PKCi promotes an immune suppressive TME that confers resistance to a-PD-1 in LUSC; drugs targeting PKCi signaling, either alone or in strategic combination, will sensitize LUSC tumors to a-PD-1; and PRKCI CNG, elevated PKCi expression and/or PKCi-dependent signaling intermediates will be useful predictive biomarkers of response of human LUSC tumors to a-PD-1. These hypotheses will be tested through completion of three interrelated specific aims. First, characterize the immune TME, identify PKCi-dependent immune suppressive signaling mechanisms, and assess a-PD-1 response in the newly developed GEMM of PKCi-driven LUSC. Second, evaluate the ability of drugs targeting PKCi immune suppressive signaling to sensitize PKCi- dependent LUSC tumors to a-PD-1. Finally, assess whether PKCi-related biomarkers predict response to a-PD1 in human LUSC tumors. Successful completion of these aims will enhance the understanding of PKCi-mediated immune suppression and a-PD-1 resistance in LUSC, develop novel therapeutic approaches to improve response of LUSC to a-PD-1, and identify better predictive biomarkers of a-PD-1 response in human LUSC patients. Clinical translation of key findings will be facilitated by the active clinical development of the PKCi inhibitor ANF.

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#:** 23B10 Restoring FZR1 Tumor Suppressor Function in Human Cancers

Principal Investigator: Lixin Wan, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: The objectives of this project are to characterize the role of fizzy-related protein homolog (Fzr1) gene as a tumor suppressor in breast cancer and possibly other types of human cancers. The hypothesis is that Fzr1 is suppressed by targetable enzymes in the tumor cells, and it is highly actionable to restore its tumor suppressor function by inhibiting these enzymes. Research staff have started to generate the reagents necessary for the proposed experiments. Genetically modified breast cancer cell lines have been made to either delete the Fzr1 gene, or ectopically express a Fzr1 mutant that could change its function in tumor cells. Furthermore, a gene targeting vector to express a Fzr1 mutant was also made, this vector will be used to target the Fzr1 gene in the mouse. Research staff found that the mice with Fzr1-deleted in their epithelial cells developed breast tumors. These tumor samples are being analyzed by histological assays to determine their breast cancer subtype. Research staff are also

preparing the samples for proteomic analysis of how the restoration of the tumor suppressor function of Fzr1 alters the proteome in triple-negative breast cancer cells.

Follow on Funding: None at the time of reporting.

Collaborations: Professor Branko Stefanovic, Florida State University, an expert in collagen synthesis biology.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

11. **Grant#:** 23B11 Modulating dendritic cell (DC) polarization and biology with L-fucose to enhance DC vaccine efficacy

Principal Investigator: Eric Lau, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: Despite reports of remarkable efficacy of immunotherapies in patients with immunologically “hot” tumors types (e.g., melanoma), those with immunologically “cold” tumors (e.g., breast cancer (BC)) do not generally exhibit significant upfront responses to immunotherapies. As immunotherapies act by harnessing a patient’s immune system to destroy their tumors, lack of efficacy can be attributed to low numbers of immune-stimulating, tumor-suppressing immune cells or high numbers of immune-suppressing, tumor-promoting immune cells inside breast tumors. Effective anti-tumor immune responses occur when the former functionally overcome the latter. Myeloid cells are immune cells that can dramatically shape the immunostimulatory vs. immunosuppressive landscape in tumors. The ability to boost tumor-suppressive functions while inhibiting tumor-promoting functions of myeloid cells would be a significant advance in the treatment of cancer; however, there are yet to be effective, specific, and safe methods to do so. The research staff recently discovered that the dietary sugar L-fucose (LF) can potently suppress tumors, in part via this exact powerful dual-action effect: simultaneously blocking the immunosuppressive capacity of myeloid cells while triggering myeloid maturation into an immunostimulatory subtype of dendritic cell (DC) known as monocyte-derived DCs (moDCs) in breast and melanoma tumors. L-fucose is the first non-toxic dietary agent, to common knowledge, that can be used to stimulate anti-tumor myeloid function and has significant implications for enhancing an emerging immunotherapy called “dendritic cell vaccine” (DCV), which leverages the anti-tumor power of DCs. In DCV, DCs are extracted from a patient’s tumor, expanded, optimized, and re-infused back into the patient. When successful, DCVs can provide durable anti-tumor immune responses and relapse-free survival. However, efficacy rates of DCVs are limited to subsets of BC patients. Poor responses are associated with low tumor infiltration with DCs, and suboptimal ex vivopropagation and function of moDCs, a main type of DC used for DCV. Overcoming these hurdles is an urgent clinical needed. The researchers propose to determine how LF regulates myeloid/DC biology and how it might be used to improve DCV efficacy. The research team will implement a series of cutting-edge approaches. The research team expects these discoveries to advance the understanding of myeloid/DC biology and to inform how LF might be used to enhance DCV and outcomes for patients with BC and other cancers.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

12. **Grant#:** 23B12 Cutting Fuel Lines to Ras Driven Cancers with Marine Natural Products

Principal Investigator: Esther Guzman, PhD

Organization: Florida Atlantic University

Summary: The overarching goal of this project is to discover marine natural products from the Harbor Branch Oceanographic Institute (HBOI) genetically encoded secondary metabolite library that can function as potential therapies targeting macropinocytosis and autophagy in Ras-driven cancers. Mutations in the oncogene Ras can accelerate tumor initiation and progression. Ras-driven tumors have developed unique ways to provide nutrients to the growing tumor. Macropinocytosis is a process by which the cell membrane ruffles to trap fluid (and nutrients within that fluid) by creating vesicles that are then internalized and processed by lysosomes. Macropinocytosis provides nutrients to tumors leading to their growth and resistance to current drugs. Autophagy is a normal cellular process activated upon low nutrient conditions to break down unnecessary or damaged proteins and cellular organelles into building blocks such as amino acids that can be used for new biomolecule synthesis and/or energy production. In cancer, once a tumor exists, autophagy has been shown to provide nutrients that promote tumor growth, chemoresistance, and survival. Early studies suggest that blocking these processes can inhibit tumor growth and provide novel modalities for treating Ras-driven cancers, which include pancreatic, triple negative breast, lung, and colon cancers. Natural products are an important source of new drugs. In nature, both sponges and corals depend on macropinocytosis as a source of nutrients and Cnidaria use autophagy to respond to starvation and stress. It can be envisioned that production of natural products that impact these pathways may have evolutionary advantages, Manzamine A, a marine sponge derived compound, can block autophagy and decrease tumor volume in animal models of pancreatic cancer. The researchers hypothesize that additional marine natural products that modulate autophagy and/or macropinocytosis can be discovered from the HBOI library. Researchers will identify these compounds through using phenotypic high content imaging assays to identify materials for their ability to modulate autophagy and/or macropinocytosis, identifying the active natural products using bioassay-guided fractionation and spectroscopic methods, and beginning to characterize the utility of the compounds discovered through confirmatory secondary assays. Successful completion of these Aims will lead to the identification of compounds with the potential to be further developed into clinically useful agents for the treatment of Ras-driven 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.

13. **Grant#:** 23B13 Translational Utility of Tumor-Derived FGF19 in a Novel Blood-Based Endocrine Suppression Approach

Principal Investigator: Deborah A. Altomare, PhD

Organization: University of Central Florida

Summary: Although breast and colorectal cancers are treatable when caught early, advanced tumors are highly lethal emphasizing the importance of routine screening. In fact, screening is the best predictor of survival with continued reduction in incidence and mortality stemming from enhanced screening compliance. However, shortcomings in screening become more apparent when health disparities are considered, as screening uptake and adherence closely depends on healthcare accessibility. There is a strong incentive to explore more accessible approaches to enhance screening. Blood-based testing represents an appealing and relatively less expensive option, although such tests have been limited by a lack sensitive and specific serum markers that are able to pinpoint malignancy from a single reading. The researchers propose a novel two-stage blood test to detect the enteroendocrine protein Fibroblast Growth Factor 19 (FGF19). This group has identified that FGF19 demonstrates unique characteristics that make it an attractive serum marker for this concept. The overarching hypothesis is that cancer-derived FGF19 in the context of a two-step suppression-based test predicated on differentiating between normal and elevated FGF19 levels is more accurate at detecting tumors than a conventional single step test. Objectives are to assay thresholds, endocrine effects of malignant FGF19, and its translational potential as a novel screening marker. Researchers will utilize human cancer cells and patient samples to test for aberrant FGF19 expression and secretion, and determine whether abnormal FGF19 levels are found in the blood and tumors of CRC and/or breast patients. Researchers will then use animal models with tumors derived from human tumor cells to test blood levels and levels of malignant FGF19, and to profile systemic tissues for pharmacokinetic response to FGF19. Researchers will also use a pre-clinical mouse model of CRC to test FGF19's translational potential as a screening marker via the proposed two-step suppression test. Successful outcomes from this proposal will provide significance for FGF19 as a novel cancer blood marker and justifiy further development of the two-stage suppression test for detecting types of cancer in a clinical context. These findings will provide rationale for assessing the clinical impact of FGF19 as a blood marker for screening and/or monitoring of breast cancer and/or CRC progression in patients, and for detection of other FGF19 positive cancers that do not have non-invasive screening options.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

14. **Grant#:** 23B14 Potentiating Immunotherapies via Polyamine Blocking Therapy

Principal Investigator: Otto Phanstiel, PhD

Organization: University of Central Florida

Summary: The objective of the project is to potentiate existing immunotherapeutics like PD1 inhibitors using polyamine blocking therapy. Polyamines (putrescine, spermidine and spermine) are low molecular weight aliphatic amines, which are mostly charged at physiological pH. Polyamines play many roles in cells including key roles in regulating translation, transcription, chromatin remodeling and immune privilege. Indeed, many cancer types upregulate polyamine biosynthesis and import in an effort to increase their supply of the native polyamines needed for growth. Spermine is made from spermidine via the activity of spermine synthase (SMS) and spermine can be secreted to generate local immune privilege surrounding the tumor. Polyamine blocking therapy (PBT) involves using a combination therapy of a polyamine biosynthesis inhibitor like difluoromethylornithine (DFMO) and a polyamine transport inhibitor (PTI). These two agents (DFMO+PTI) cause polyamine depletion as both polyamine biosynthesis and transport are blocked. This in turn leads to lower levels of the native polyamine spermine. Lowering the levels of the natural immune suppressant spermine provides an opportunity to boost the efficacy of immunotherapeutics. The idea is that human cancers use their high polyamine content to make spermine which is secreted to protect them from the immune system. This is actually a fetal strategy, where the fetus is protected from maternal antibodies via sustained levels of spermine in the amniotic fluid. Research staff hypothesize that certain cancers are using this fetal strategy to avoid immune clearance. Immunotherapies have failed in many human cancers and the hypothesis is that immunotherapies will continue to fail until the spermine shield is addressed. This project seeks to provide a solution to this clinical problem by developing a combination therapy of PBT and an immunotherapeutic. The PBT will lower the spermine shield and the immunotherapeutic will potentiate the immune repsonse to help clear the tumor. Several methods will be used including organic synthesis of the PTI agents, HPLC determination of polyamine levels inside cells, cell culture methods, histology, immune cell profiling and animal models of human cancers to assess how well the combination therapies work in vivo. A success here could provide new hope in the form of a combination therapy which converts recalcitrant tumors into responsive tumors by lowering the natural spermine shield.

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.

15. **Grant#:** 23B15 Notch1 selective targeting to overcome immunotherapy resistance

Principal Investigator: Barbara Bedogni, PhD

Organization: University of Miami

Summary: Immunotherapy has made great strides in the treatment of several cancers, most of all melanomas. The combination of the immunocheckpoint inhibitors (ICIs) pembrolizumab or nivolumab with ipilimumab, is standard of care for advanced melanoma. With this combination the five-year survival rate is 50% However, the combination therapy can be highly toxic and still does not provide efficacy for half the patient population whose melanomas are resistant. A main caveat for immunotherapy success is the presence of an ideal tumor immune microenvironment (TiME) in which tumor cells reside. This TiME is enriched in anti-tumorigenic immune cells, such as cluster of differentiation 8 (CD8)+T cells, and an overall “inflamed” condition with the

presence of molecules such as Interferon-gamma, that can activate CD8+T cells to fight the tumor cells. However, patients that do not respond to ICIs (non-responders), tend to present a so called “cold” TiME, which is depleted of such factors and cells and enriched in cells and molecules that instead favor tolerance, a condition that promotes tumor development and progression. Thus, ways to favor an “inflamed” TiME in melanoma would promote immunotherapy efficacy by sensitizing non-responders to the treatment. The researchers plan to do so by using a novel anti Neurogenic locus notch homolog protein 1 (Notch1) antibody researchers have produced. Notch1 is an evolutionarily conserved molecule that controls key pathways involved in embryogenesis and adult tissue repair. In normal adult tissue Notch1 levels are usually low, however, several cancers highjack the Notch1 pathway to gain growth and survival advantages. In melanoma, researchers have previously shown that 60% of tumors express high levels of active Notch1. Importantly, the research staff showed that Notch1 not only promotes tumor intrinsic features such as growth and survival; but it also hinders the inflamed TiME. High levels of Notch are in fact associated with non-responders to ICIs. Thus, the researchers propose to specifically target Notch1 to induce an inflamed TiME to increase ICI efficacy against melanoma and revert resistance. The data shows that the anti-Notch1 antibody is very selective towards Notch1 without interfering with other Notch factors, causes melanoma cell death with no deleterious effects on normal cells, is not toxic in vivo, delays melanoma tumor growth, and promotes an “inflamed” TME enriched in CD8+ T cells and molecules, such as Interferon-gamma, that promote CD8+ T cells anti-tumor activity. Goals of this proposal are to determine the efficacy of anti-Notch1 antibody in combination with immunotherapies, administered either alone or in combination in responders and non-responders melanoma mouse models, investigate the anti-tumor mechanisms that Notch1 neutralization exerts both on the tumor cells specifically, as well as on the TiME, address the prognostic potential of Notch1 in melanoma patients. The overarching goal of this proposal is to identify novel therapeutic modalities to improve the efficacy of immunotherapy for metastatic melanoma patients and therefore their survival.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

16. **Grant#:** 23B16 Next-Generation Biomedical Big Data Platform for Cancer Research and Collaboration Across Florida

Principal Investigator: Stephan C. Schurer, PhD

Organization: University of Miami

Summary: Cancer research has increasingly become data-driven: the advent of high-throughput multi-omics technologies has led to the generation of large volumes of diverse biomedical data and has transformed cancer research into a computationally demanding science. This large-scale generation of biomedical data necessitates new strategies for efficient data management and analysis, to accelerate the development of next-generation precision medicine solutions, including cancer prevention strategies and new targeted treatments. Additionally, current cancer health disparities based on race, ethnicity, and socioeconomic

status could be reduced if available data were better utilized. A significant need exists for computational infrastructures that will permit such integrative analyses, and Florida cancer researchers would benefit from such a platform. In the proposed research infrastructure project, researchers will enhance and deploy the Big Data bioinformatics platform across six Florida research institutions to advance cancer research and precision medicine. The foundation for this state-wide infrastructure will be the Sylvester Data Portal (SDP, https://sdp.miami.edu), the internal bioinformatics platform developed at the Sylvester Comprehensive Cancer Center (SCCC) facilitating the processing, findable, accessible, interoperable and reusable (FAIR) data management, bioinformatics analysis, secure sharing, and long-term storage of preclinical and clinical data generated at SCCC, while adhering to Florida state law and federal privacy regulations. The first aim of the project will involve creating the regulatory and data privacy framework that will govern the state-wide cancer research network. The second aim will establish and deploy a cancer research bioinformatics infrastructure across the six collaborating Florida institutions by expanding the Sylvester Data Portal. To facilitate the rapid development and deployment of new functionalities and bioinformatics tools across the Florida organizations, researchers will develop a Software Development Kit (SDK) that will enable researchers to create customized applications integrated with the data ecosystem. In the third aim, researchers will demonstrate the potential of this novel research infrastructure to accelerate cancer research by integrating and harmonizing diverse biomedical datasets and deploying novel bioinformatics capabilities across this Florida research network. Additionally, bioinformatic resources to mitigate existing health disparities in cancer treatment and outcomes will be developed. Via this novel research informatics platform, resources will be shared among the collaboration network, and selected resources will also be shared with the broader cancer research community. The proposed research infrastructure platform will be a valuable asset to enhance Florida's cancer prevention and treatment strategies, and precision medicine landscape, and establish new commercialization opportunities.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix B: William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program Fiscal Year 2022-2023 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, Inc.	Susan Vadaparampil, PhD	$1,424,806.00	9/30/26	No	No	No
22B02	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Eric K. Lau, PhD	$573,000.00	11/30/25	No	No	No
22B03	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Jose R. Conejo-Garcia, MD, PhD	$1,432,499.00	9/30/26	Yes	No	No
22B04	Relinquished						
22B05	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Joseph Kissil, PhD	$573,000.00	3/31/25	No	No	No
22B06	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Brandon Manley, MD	$716,250.00	9/30/26	No	No	No
22B07	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Vincent Luca, PhD	$573,000.00	3/31/25	No	No	No
22B08	Mayo Clinic Jacksonville	E. Aubrey Thompson, PhD	$573,000.00	3/31/25	No	No	Yes
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	Yes	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, Inc.	Alvaro Monteiro, PhD	$200,000.00	5/31/24	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, Inc.

Summary: 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 one-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 to test whether HPV MISTICS increases HPV vaccine initiation and completion rates in adolescents aged 11-17, explore covariates of intervention effects, and explore equity of implementation outcomes and identify implementation barriers and facilitators. The study team has gained

approval by H. Lee Moffitt Cancer Center and Research Institute, Inc. Scientific Review Committee and Institutional Review Board 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, Inc.

Summary: Melanoma, one of the deadliest cutaneous malignancies, can rapidly spread and evade the immune system. Survival probabilities plummet once melanomas metastasize from the primary tumor site. Incidence and mortality rates for melanoma are historically higher for men than women, with around 50% more new cases and twice the lethality in men in the United States in 2020. Although underlying mechanisms are unclear, melanomas can express androgen receptor (AR) and respond to androgen, which elicits tumor-promoting effects. Thus, the therapeutic inhibition of AR represents an attractive and potentially paradigm-changing treatment strategy for melanoma, given the availability of clinically approved AR antagonists (ARAs) for prostate cancer. However, ARAs are not without significant quality-of-life-compromising effects. Unfortunately, there is a lack of mechanistic insight and specific biomarkers that are essential for delineating which patients would benefit from administration of ARAs and what therapeutic modalities might be enhanced by co-administration of ARAs. Thus, elucidation of androgen-/AR-regulated mechanisms of melanoma biology is urgently needed. In this proposal, researchers will elucidate a novel, key molecular mechanism underlying androgen/AR-stimulated melanoma invasiveness and metastasis. The research team recently discovered a connection between sex and melanoma fucosylation, the modification of proteins with the sugar L-fucose (L-fuc). Fucosylation can promote or suppress tumors—divergent functions that are dictated by 13 tumor-promoting or tumor-suppressing fucosyltransferases (FUTs). In melanomas in men, global fucosylation is reduced. The research team found that androgen/AR reduces global fucosylation levels while increasing a form of fucosylation mediated by tumor-promoting FUT4, potently driving melanoma invasiveness. In this application, researchers will test the hypothesis that androgen/AR drives melanoma invasiveness by inducing FUT4-mediated regulation of AJs. In this grant, researchers propose to: (i) delineate how AR-FUT4 regulates AJs and invasiveness and how that contributes to melanoma metastasis, and (ii), assess how this insight can be leveraged as diagnostic/predictive biomarkers and to enhance targeted and immunotherapies.

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, Inc.

Summary: Small Cell Lung Cancer (SCLC) continues to be a major area of study in recent years at Moffitt Cancer Center and Research Institute, Inc. (Moffitt). The original proposal remains focused on three key aims: (1) Define the Immunogenic Intra- and Inter-Tumor Heterogeneity of human small cell lung cancer. (2) Elucidate the Trajectory of Differentiation of tumor-reactive T cells in small cell lung cancer. (3) Design Cellular Therapies that target heterogenous metastatic disease. The research team has made significant progress with completion of ribonucleic acid (RNA)-Sequencing and deoxyribonucleic acid (DNA) sequencing of small cell lung cancer samples from different metastatic masses sourced from autopsies of SCLC patients. The data that the researchers harvested and continue to analyze is pivotal to the future steps of the study to define the heterogeneity of SCLC and identify novel therapeutic targets. A few significant immunotherapeutic targets have emerged, and the research team is continuing to pursue these targets in the coming year. Among these targets that researchers are considering pursuing further, the team is most excited about ADAM20, transmembrane Protein 191C (TMEM191C), and taste receptor type 2 member 13 (TAS2R13). The research team is targeting these surface-expressed proteins for further development of single-chain variable fragments (scFvs) and novel CAR-T targets. Researchers are continuing to evaluate variability in areas of immune infiltration, specifically planning for development of CAR-T targets with both alpha/beta and gamma/delta T Cells. A significant thrust area is the examination of gamma/delta T cells from SCLC samples and other cancer types including non-small-cell lung carcinoma (NSCLC) and high grade serous ovarian cancer. As researchers better understand the behavior and composition of these cells, the aim is to refine and develop the most effective therapeutic strategies. As research moves into the next phase, the hope is to continue to collect samples and translate laboratory findings into effective therapeutic strategies with long term plans for testing and validating in clinical settings, to improve outcomes for patients with SCLC.

Follow on Funding: Conejo-Garcia, J, Perez, B, PI, NIH-NCI R01, 04/01/2023 - 03/31/2028. Total Funds Requested: $2,833,921. Submission Date: 06/05/2022. Pending.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

4. **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, Inc.

Summary: Current treatment strategies for lung cancer are dependent on tumor subtype and stage. Despite recent progress in treatment, the five-year survival rate for non-small cell lung cancer (NSCLC) is 24%. One of the most promising directions in treatment for lung cancer has been the development of targeted therapies that are designed to target specific molecular alterations in proteins that are required for the growth and/or survival of tumor cells. These therapies stem directly from research efforts aimed at understanding the molecular mechanisms that drive lung tumorigenesis. In a recent example, drugs the research team developed to target Kirsten rat sarcoma (KRAS) G12C, which is an oncogenic mutation found in 13% of NSCLC patients. Clinical trials are currently underway to assess the use of KRASG12C inhibitors as single agents and in combination with other drugs. The development of KRASG12C inhibitors has raised the possibility that other oncogenic alleles of KRAS could be targeted. Moreover, several lines of evidence suggest that different KRAS alleles are associated with distinct clinical behaviors and responses to treatment. Researchers' long-term goals are to understand the functions of different KRAS alleles and identify specific vulnerabilities that can be exploited for therapeutic gain. Towards this goal, researchers will focus on mutations in KRAS using a newly developed allelic series of isogenic lung adenocarcinoma cells. The term "isogenic" refers to the fact that the cell lines being used differ only in the status of KRAS, thus removing other confounding factors. The isogenic cells panel will allow researchers to carefully characterize and compare the effects of these mutations at a cellular level and follow changes in the signaling events downstream of the different oncogenic KRAS alleles in the isogenic cells. Over the past year, the researchers have been characterizing how the different alleles confer differences in rates of proliferation in the cells. Researchers also found changes in the molecules that relay the effects of the mutated KRAS protein in the cells. With these early discoveries the research team is moving forward as planned with a more extensive analysis using novel transcriptomic and proteomic approaches.

Follow on Funding: None at the time of reporting.

Collaborations: None of at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

5. **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, Inc.

Summary: Since the start of the study in April 2022 the researchers have made significant progress in executing the outlined proposal. The research study is seeking to investigate the possible role of unique splice variants present in the ribonucleic acid (RNA) of patients with kidney cancer, specifically clear-cell renal cell carcinoma, as a clinical biomarker. Over the last 15 months the researchers have been able to accrue a total of 88 patients with clear-cell renal cell carcinoma for the study. For a subset of the samples thus far the research team have begun extracting RNA to determine the best methods for each sample type (i.e. blood, tumor, urine). During this process the researchers have conducted quality control experiments to minimize noise from nonspecific deoxyribonucleic acid (DNA) and RNA nucleotides that may convolute detection of the specific RNA splice variant targets. The researchers have adjusted its protocol to enrich the RNA extraction for downstream analysis.The research staff have also begun testing the sample RNA using a platform called nCounter that allows the research team to specifically quantify splice variant targets in the patient samples. Initial use of this platform as testing assay has allowed the research team to optimize conditions for analysis of this data to increase the sensitivity and specificity of the assay. Researchers have conducted two rounds of testing each with 12 patients' samples thus far. The first round was done with patient's blood before surgery along with the patient's matched clear-cell renal cell carcinoma tumor. The goal is to identify a robust "signal" for each respective RNA splice variant and the variance of each spice variant across a set of six patients with proven clear-cell renal cell carcinoma. This assay also allowed the research team to begin optimization of its bioinformatics analysis for data obtained from this platform. The second round of testing involved primarily using healthy controls so that researchers could identify baseline levels of detection for each splice variant among patients without a diagnosis of clear-cell renal cell carcinoma. These results have served the purpose of helping the research team establish the expected "noise" that may be anticipated among all patient samples used for this study. Learning from these two initial rounds of testing on the nCounter platform, the researchers have further optimized a larger panel of 48 patients' samples including matched blood and tumor samples before and after surgery (three samples per patient) to begin looking at patient-specific profiles for the splice variant RNA targets. This experiment is currently underway.

Follow on Funding: None at the time of reporting.

Collaborations: None of at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

6. **Grant#:** 22B07 Structure-guided engineering of LAG3 immunomodualtory function

Principal Investigator: Vincent Luca, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: The goal of this proposal is to determine how the cancer drug target, Lymphocyte Activation Gene 3 (LAG3), prevents the immune system from killing tumors. Under normal conditions, LAG3 functions like an "off-switch" that prevents the immune system from overreacting to an infection. However, melanoma cells often hijack LAG3 to turn off T cells and evade clearance by the immune system. The United States Food and Drug Administration (FDA) recently approved the LAG3 inhibiting antibody, realtime, for the treatment of advanced

melanoma, ushering in a new wave of efforts to generate the maximally effective LAG3-targeting drugs. In this proposal, the research team is pursuing three aims to try and accelerate the development of new LAG3-basd therapies. The first aim is to visualize how LAG3 proteins on the surface of T cells interacts with tumor cells on the atomic scale. The second goal is to identify vulnerabilities in the LAG3 molecule that can be targeted with inhibitory antibodies. The third goal is to engineer LAG3 “decoy” proteins that can block the function of naturally occurring LAG3. During the first year of funding, the research team made progress on all three aims. For the first aim, a major challenge was the isolation of purified LAG3 proteins bound to tumor molecules. To solve this problem, the team used molecular engineering to tether LAG3 to its binding partner, Major Histocompatibility Complex II (MHCII). These engineered molecules will mimic the interaction that occurs between LAG3 on T cells and MHCII on tumor cells. Subsequent microscopy studies revealed that the LAG3-MHCII complex was flexible and will require further stabilization for optimal imaging. Once the complex is fully stabilized, the team will determine a near-atomic resolution structure to identify “hot spots” on the LAG3 molecule that can be targeted with drugs. For the second Aim, the team isolated a panel of nine miniaturized antibodies (called nanobodies) that bind to LAG3. The nanobodies each bind to different regions of the LAG3 molecule and may have unique therapeutic properties depending on the site nanobodies recognize. The team’s next goal is to test how these nanobodies block the ability of LAG3 to turn T cells “off”. Once the most potent nanobodies are identified, the team will map the binding site on LAG3 to identify a key vulnerability that can be exploited in drug development. For the third Aim, the team engineered mutant LAG3 proteins that bind to MHCII more tightly than natural LAG3. These LAG3 decoys were synthesized and are currently being tested for their ability to affect immune cell function. Future studies will assess how strongly the decoys block LAG3 and will closely examine the effect of the decoys on immune cell function.

Follow on Funding: None at the time of reporting.

Collaborations: None of at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

7. **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

Summary: Triple negative breast cancer (TNBC) is an aggressive disease with a high mortality, particularly in young women and women of African/American descent. It is understood that there is an immune component to therapeutic response in TNBC, but the fundamental principles that impinge on interaction between host immune cells and tumor cells are largely unknown. This is a matter of considerable clinical significance, since almost all TNBC patients are treated with immune checkpoint inhibitors (pembrolizumab). Only a subset of TNBC patients benefit from this treatment, which is expensive, and which has significant side effects. The unmet clinical needs are therefore to identify patients who are likely to benefit from this treatment and to understand why most patients do not respond. Such an understanding is critical to the development of novel therapeutic strategies to overcome resistance to immune checkpoint

inhibitor therapy. The team is developing and deploying novel, cutting edge single cell spatial transcription profiling technology (NanoString CosMx Spatial Molecular Imaging) to explore the relationship between clinical phenotype (i.e., therapeutic response) and the number, types, activities, and locations of immune and other cell types within TNBC tumors. An initial analysis indicated that outcome was closely linked to the process known as antigen presentation. This process is central to the mechanism whereby immune cells recognize and kill tumor cells, and the team's published data reveal that tumor cells in some patients are defective in this process, thereby precluding their recognition by the host immune system. During the first year of this study, the team has used CosMx technology to interrogate an initial subset of TNBC tumors with a view towards identifying the key antigen-presenting cells and quantifying all relevant immune cells that are interacting with tumor cells in a manner that might influence tumor cell antigen presentation. Preliminary data strongly suggest that tumor cell antigen presentation (and therapeutic outcome) is primarily dependent upon a subset of rare plasmacytoid dendritic cells (pDCs). The goals in the second grant year include orthogonal validation of the role of antigen presentation as a biomarker for predicting response to standard of care immune checkpoint inhibition, confirmation of the role of pDCs in antigen presentation and therapeutic response, and identification of additional features that are associated with therapeutic response and might therefore inform the mechanism of therapeutic resistance and/or offer attractive targets for alternative therapies.

Follow on Funding: None at the time of reporting.

Collaborations: Dr. Malu Tansey, University of Florida, is collaborating with the research team on a project related to the immune architecture of inflammatory bowel disease. More relevant to the aims of the Bankhead-Coley Cancer Research Program (Bankhead) grant, the researchers have initiated a collaboration with a group of investigators at Emory University Medical School. Headed by Dr. Zachary Buchwald, this group has a cohort of TNBC samples treated with pembrolizumab. Researchers have agreed to analyze these samples, which will provide an outstanding validation cohort for the Bankhead studies. The ability to validate the findings in an independent sample cohort adds tremendous power to the analyses.

Journals: Carter JM, Chumsri S, Hinerfeld DA, et al. Distinct spatial immune microlandscapes are independently associated with outcomes in triple-negative breast cancer. *Nat Commun*. 2023;14(1):2215. Published 2023 Apr 18. doi:10.1038/s41467-023-37806-0.

Patents: None at the time of reporting.

8. **Grant#:** 22B09 Spliceosomal modulation for regulation of melanoma immunogenicity

Principal Investigator: Dmitriy Minond, PhD

Organization: Nova Southern University

Summary: As estimated by the National Institutes of Health 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 over 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 heterogeneous nuclear ribonucleoprotein (HhnRNPH1) 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: determine role of H1/H2 in melanoma immunogenicity in vitro; determine role of H1/H2 in melanoma immunogenicity in vivo; and 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 (NSU) bring 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.

9. **Grant#:** 22B10 Mitochondrial modulators of multiple myeloma growth and therapy resistance

Principal Investigator: Jonathan Licht, MD

Organization: University of Florida

Summary: Chromosomal translocation between chromosome 4 and chromosome 14, found in 15% of multiple myeloma (MM), leads to overexpression of histone methyltransferase nuclear receptor binding SET Domain Protein 2 (NSD2) which drives an oncogenic program and poor prognosis. Identification of genes required for MM cell grow can lead to develop novel therapies. Researchers found that loss of the gene encoding adenylate kinase 2 (AK2) genes is more detrimental to NSD2 high cells. AK2, localized in the mitochondrial intermembrane space, generates adenosine triphosphate (ATP) which is the major energy carrier in the cell. High antibody production MM puts cells under endoplasmic reticulum (ER) stress, increasing the need for energy (ATP) to fold proteins.AK2 is required to resolve ER stress in MM cells, likely due to its ability to generate ATP. AK2 overexpression is linked to MM resistance to proteasome inhibitors (e.g., bortezomib), therapies that kill MM by generating ER stress. The researchers hypothesize that MM growth depends on mitochondria energy production and AK2 to prevent ER stress. The aims of this project are to define the molecular basis of the dependence of NSD2 high MM on AK2. Results so far show that loss of AK2 leads to an increased load of unfolded proteins in NSD2 high but not NSD2 low cells, which may a basis of the greater sensitivity of NSD2 high cells to AK2 inhibition. Furthermore, AK2 depletion enhanced the activity of the anti MM therapy agent bortezomib which works by increasing unfolded proteins, thus representing a new therapeutic concept. The lab investigated the effect of AK2 depletion on gene expression in KMS11 NSD2 high and NSD2 low cells by RNA expression profiling. Research staff observed a stronger transcriptional response to AK2 loss in NSD2 high cells. Further investigation revealed that AK2 depletion in NSD2 high but not NSD2 low cells lead to

DNA damage, due to replication stress, meaning that the cell is creating errors when replicating its DNA. Analysis of metabolites in the cell showed that there was a deficit of deoxyribonucleotides (these are the building blocks of DNA) in NSD2 high cells upon loss of AK2; this accounts for the errors and DNA damage during replication because treatment of the cells with extra deoxyribonucleotides rescued the cells from DNA damage and cell death. Researchers will also, investigate the role of AK2 and other mitochondrial constituents in MM fitness and therapy response within the bone marrow microenvironment. The role of AK2 will be assessed in vivo using the MOPC315.BM mouse model which the lab recently obtained as well as other human and mouse cell lines.

Follow on Funding: Licht, JD, PI. Leukemia Lymphoma Society Translational Research Program. 8/1/22-7/31/25. Submission Date: 01/15/2022. Total Funds Awarded: $200,000.

Collaborations: Collaborations
Laboratory of Navdeep Chandel, PhD - Northwestern University, Department of Medicine, Chicago, IL - has performed metabolomics studies for the Licht lab determining how down-regulation of AK2 changes the abundance of metabolites in multi0okle myeloma cells.
Laboratory of Baek Kim, PhD Emory University to measure deoxynucleotides in AK2 deficient myeloma cells.

Training University of Florida Undergraduates: Greeshma Surapaneni, Alisha Patel, Gabriella Ospina, Nofel Iftikhar

Journals: None at the time of reporting.

Patents: None at the time of reporting.

10. **Grant#:** 22B12 Targeting mitochondrial protein synthesis to combat blood malignancies

Principal Investigator: Antonio Barrientos, PhD

Organization: University of Miami

Summary: 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 cluster of differentiation (CD) (CD34+CD38-) are resistant to targeted signaling inhibitors, such as the breakpoint cluster region (BCR)/ABL kinase class of inhibitors, often a problematic source of resistance leading to minimal residual disease. Tigecycline, a United States Food and Drug Administration (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 OXPHOS. The main goal of this proposal is to determine the mechanism by which elatol inhibits mitochondrial translation and its usefulness in targeting mitoribosomes as a therapeutic strategy against several types of leukemia. Researchers hypothesize that the dependence of leukemia cells on OXPHOS makes them especially vulnerable to inhibition of mitochondrial protein synthesis. In this year research results have shown that leukemia cell lines have a mitochondrial respiratory chain complex organization different than that control cells, including an increase in CIII2-CIV supercomplex

levels. Researchers have also found that increased mitochondrial biogenesis is also reflected in increased mitochondrial dioxyribonucelic acid (mtDNA) levels. Researchers have obtained promising data showing that the protein leucine-rich pentatricopeptide repeat -motif-containing protein (LRPPRC), a mitochondrial mitochondrial ribonucelic acid (mRNA) stablization and translation factor, could be the elatol target in mitochondria. Elatol-induced mitochondrial translation inhibition triggers activating transcription factor 4 (ATF4) activation, leading to the induction of the integrated stress response (ISR). When ISR is persistent, it induces cell death. 4-Elatol specifically kills cancer cells by apoptosis, and at least in two cell lines, ferroptosis is also involved. With the improvement of the fundamental understanding of the pathways used by leukemia cells to meet their higher energy, metabolic, and signaling demands, the researchers are uncovering the LRPPRC-mediated mechanism by which the marine natural product elatol blocks mitochondrial protein synthesis and elicits the integrated stress response, and are advancing in the pathways that promote cell death in several types of leukemia prevalent in the population.

Follow on Funding: None at the time of reporting.

Collaborations: Collaboration with the laboratory of Dr. Alexey Amunts (Scilife Lab, Stockholm University). Dr. Amunts is helping University of Miami by applying cryo-electron microscopy to solve the structure of elatol-bound mitochondrial ribosome. One PhD student from that laboratory (Vivek Singh) is assisting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

11. **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

Summary: New treatments are needed for patients with diffuse large B-cell lymphoma (DLBCL), an aggressive blood cancer diagnosed in nearly 3,000 Floridians annually. Research staff 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. Preliminary data showed that exposing DLBCL cells to a GAK inhibitor halted 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 DLBCL tumors derived from them grow aggressively in patients but also are especially dependent on proteins that carry out cell division. During the first year of funding, research staff have accomplished key goals of the project including assessing the potential of GAK inhibition more broadly in DLBCL and demonstrating that the high-risk activated B-cell (ABC) subtype of the disease is most sensitive. Researchers have also demonstrated that GAK inhibition alone is highly effective, showing no increased efficacy from the addition of chemotherapy drugs or other cell cycle kinase inhibitors. This means GAK inhibition as a single modality is likely to have strong single-agent activities, pending assessment in animal models. The researchers have also achieved synthesis of two structurally distinct groups of novel GAK inhibitors with confirmed on-target inhibition of the

enzyme. This has permitted key progress toward development of a sophisticated understanding of structure-activity relationships to the target, facilitating further development of rationally designed compounds. Researchers have established that GAK forms specific structures with microtubules during cell division and that GAK inhibition leads to an abnormal accumulation of these structures. These researchers therefore reveal GAK as a new target with several advantages, including its unique activities during cell division, its highly drug-targetable kinase 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 opportunity to develop novel approaches for the treatment of DLBCL and, in the longer term, potentially additional malignancies as well. In particular, these investigators have 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.

Follow on Funding: None at the time of reporting.

Collaborations: The University of Miami Miller School of Medicine was the site of all project activities during the reporting period, including work in the laboratory of Primary Investigator (PI) Dr. Schatz, the co-PIs Drs. Feng and Al-Ali, and collaborator Dr. Schürer. One student (Austin Newsam in the Schatz lab) is performing research and receiving training as part of the project. In addition, there is a research associate position partially funded on the project. For the first two months of the award, this position was filled by Preet Kumar. This position is now filled by Olivia Lightfuss. Finally, the post-doctoral position (in Dr. Feng's group) was held by Dr. Anirban Ghoshal, PhD, who had to leave the project in February due to a family emergency.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

12. **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, Inc.

Summary: During the past year the research team conducted several preliminary experiments for both aims of the project. In order to determine the landscape of interactions of WD Repeat Domain 43 (WDR43), the research team obtained the full length WDR43 fused to galactokinase4 (GAL4) deoxyribonucleic acid (DNA) binding domain in the pGBKT7 vector. Research staff confirmed the sequence, verified it did not lead to toxicity in yeast, and confirmed that it did not auto-activate. Researchers then conducted two independent yeast two-hybrid screens against a human normalized all-tissue library. However, clones obtained in these screens corresponded to out-of-frame clones. The research team determined that the expression in yeast was too low preventing an optimal screen. To circumvent this problem, researchers have decided to split the gene in two parts (an N- and a C-terminal fragment) and conduct the screens again. Research staff have designed oligonucleotides and are now in the process of cloning these fragments in the pGBKT7 vector. To determine the role of WDR43 expression on estrogen receptor (ER)-negative mammary gland cells researchers have transfected cells with CRISPR constructs to attempt the disruption of the endogenous WDR43 gene in MCF1OA. In the first attempt, researchers could not obtain viable clustered regularly

interspaced short palindromic repeats (CRISPR) clones, which may suggest that WDR43 is essential in MCF10A. Research staff are now in the process repeating these experiments and applying an alternative approach using stable short hairpin ribonucleic acid (shRNA) -mediated silencing. These clones are currently under selection.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix C: William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program Fiscal Year 2022-2023 Active Grants Funded Fiscal Year 2020-2021

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
21B01	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Kenneth Y. Tsai, MD, PhD	$530,900.00	4/30/24	No	No	No
21B02	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Brian Czwmiecki, MD, PhD	$1,327,721.00	4/30/26	No	No	No
21B03	University of Miami	Thomas Malek, PhD	$530,880.00	4/30/24	No	No	Yes
21B04	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Florian Karreth, PhD	$530,880.00	6/30/24	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, Inc.	Gina DeNicola, PhD	$530,880.00	4/30/24	No	No	No
21B07	University of South Florida	Rex M. Philpot, PhD	$528,130.00	4/30/24	No	No	No
21B09	H. Lee Moffitt Cancer Center and Research Institute, Inc.	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	4/30/24	No	No	Yes
21B12	H. Lee Moffitt Cancer Center and Research Institute, Inc.	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, Inc.

Summary: 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 neuroblastoma rat sarcoma (NRAS). This subset of melanoma is generally less responsive to treatment so that immunotherapy 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 researchers that inducing paradoxical ERK activation in established RAS-mutant cancers, might elevate ERK signaling enough to trigger oncogene-induced senescence. The 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, C57BU6 mouse model of NRASQ61 R-driven melanoma, BRAFi-induced ERK hyperactivation synergizes with anti-programmed cell death protein 1 (PD1) to induce tumor regression, accompanied by peritumoral cluster of differentiation (CD)8+T-cell infiltration and activation and reduction of myeloid suppressor cells. Progress so far 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 previously unexposed cells. These clearly implicate T-cells and myeloid cells and give researchers several avenues to further enhance how these drugs can improve responses to immunotherapy. Researchers have also deployed several techniques to show that the key CD8+T-cells which kill tumor cells are activated significantly only combination treated 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.

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, Inc.

Summary: The research team initiated clinical trial in Stage IV human epidermal growth factor receptor 2 (HER2) patients giving priming dose of intranodal conventional type I dendritic cells (cDC1) pulsed with HER2 combined with the immune stimulant Pepinemab to drive anti-HER2 cluster of differentiation 4 (CD4) T helper cell expansion in the blood and then expand those CD4 T helper cells ex vivo to transfer back larger numbers to patients with cDC1 and Pepinemab. The first low dose cohort is complete with two patients demonstrating stable disease and improved symptoms and two patients, one reaching stable disease but progressing and the last patient having progressivism disease. The treatment has been noted to be safe with only minimal fevers and chills. Researchers will start the higher dose treatment as the next phase of the study. The research team in the laboratory has made the significant observation that there are actually two CD4 populations activated by the cDC1. One is the CD4 T helper cell being grown here but there is also a hybrid cell called a natural killer/T cell that is also CD4 in the mice and this cell is also significantly increased in tumors responding to cDC1 therapy. Researchers are hotly testing to see if this cell maybe more effective in the metastatic cell killing as it offers multiple ways in which it can eliminate HER2 cancer cells. This cell is much less studied in cancer but appears to play a role in breast involution following pregnancy so researchers believe this cell may play a prominent role in mediating anti-breast cancer responses and has not been tested in patients with breast cancer as little is known about its role in breast cancer. The research team believes this may the tip of an iceberg that unlocks new opportunities for immune based therapies in breast cancer. Its role in preclinical breast cancer will be intensely in the next phase of the study.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

3. **Grant#:** 21B03 CD4+ T effector cells in cancer immunotherapy

Principal Investigator: Thomas Malek, PhD

Organization: University of Miami

Summary: The overall goal of the project is to assess a novel interleukin-2 (IL-2) analog, IL-2/cluster of differentiation (CD)25, 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. 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 KM, Malek TR. Engineering IL-2 for immunotherapy of autoimmunity and cancer. *Nat Rev Immunol*. 2022;22(10):614-628. doi:10.1038/s41577-022-00680-w.

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, Inc.

Summary: Over the last year, H. Lee Moffit Cancer Center and Research Institute, Inc. (Moffitt) focused research efforts on characterizing the signaling pathways that phosphatase and tensin homolog (PTEN) inhibits to suppress melanoma. The research team performed a drug screen in

PTEN-deficient and PTEN-restored cells to evaluate differential sensitivities. This approach demonstrated that the protein kinase B (AKT)/ mammalian target of rapamycin (mTOR) pathway is critical downstream of the lipid phosphatase activity of PTEN, which is the main tumor suppressor activity of PTEN in melanoma. Researchers had also performed a phospho-proteomics experiment that revealed several candidates downstream of the lipid phosphatase function of PTEN. Thus, the research team sought to understand how repression of the AKT/mTOR axis by PTEN suppresses melanoma. Research staff argued that critical effectors would not only be deregulated at the protein level but would likely also display alterations in RNA expression. The research team analyzed the expression of the candidates from the phospho-proteomics experiment in nevi and melanoma and found that the FOSL1 mitochondrial ribonucleic acid (mRNA) encoding the transcription factor Fos-related antigen1 (FRA1) is the most deregulated mRNA. Interestingly, FRA1 had been shown to be able to transform melanocytes, suggesting that FRA1 may be an oncogenic factor that is suppressed by PTEN. Researchers then analyzed FRA1 expression in murine and human melanoma cells and found frequent upregulation. FRA1 expression is suppressed by both inhibition of AKT and inhibition of mTOR, validating that it is downstream of this signaling axis. FRA1 phosphorylation regulates its stability, but the research team found that PTEN does not affect FRA1 turnover. Instead, research staff observed that PTEN dampens the mTOR-mediated translation of FRA1, which was rescued by expression of activated AKT. Researchers next tested if forced FRA1 expression negates the suppressive effects of PTEN restoration similar to expression of activated AKT. Interestingly, FRA1 phenocopied the effects of AKT, restoring soft agar growth, invasion, and tumor growth in allografts in PTEN-restored cells. Thus, PTEN suppresses melanoma in part by inhibiting the AKT/mTOR axis to reduce the levels of FRA1. The research team ovserved that other aspects of melanoma biology such as proliferation appear to be independent of FRA1 and to some extent also AKT. The researchers will further study the AKT/mTOR/FRA1 axis to better understand its role in melanoma metastasis.

Follow on Funding: None at the time of reporting.

Collaborations: None of 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

Summary: Cachexia, for which there is currently no medical therapy, 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. Ursolic acid is a natural compound derived from several edible herbs and fruits, including rosemary and apples, that has been shown to reduce muscle atrophy in various rodent

models but, to common 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. During this grant, the research team have tested the ability of ursolic acid to counter muscle and fat wasting in six pre-clinical models using mouse or human colon cancer cells, mouse or human pancreatic cancer cells, and mouse or human 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 against muscle tissue wasting provided by ursolic acid extends to protection against muscle fiber atrophy across all muscle fiber types and is independent of any effect on tumor growth and independent of the circulating inflammatory/cytokine profile. Thus, the protection appears to be at the level of the muscle itself. In support of this, the research team found that treatment of muscle cells with factors secreted from cancer cells causes wasting in the absence, but not the presence, of ursolic acid. To test whether the protection afforded by ursolic acid in mice with cancer carries over to when chemotherapy treatment is added, the research team treated mice bearing either colon or pancreatic tumors with 5-fluorouracil (5-FU). The team discovered that when mice with cancer are treated with 5-FU they lose significant body weight over the next 24 hours, but that mice with cancer treated with 5-FU and ursolic acid do not. These findings are encouraging in both the extent of and 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 continue to explore the biological mechanisms associated with the protection provided by ursolic acid.

Follow on Funding: None at the time of reporting.

Collaborations: None of 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, Inc.

Summary: Lung cancer accounts for the largest number of cancer-associated deaths in the state. 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 results to date suggest that quinolinic acid, a precursor for pyridine nucleotides synthesis increased in circulation by the aging process, may contribute to lung tumorigenesis independently of its

function as a precursor for pyridine nucleotide synthesis. Moreover, researchers have also uncovered the rate-limiting enzyme in quinolinic acid's metabolic pathway to be a metabolic vulnerability of lung cancer cells whose deficiency causes cell death independent of its catalytic function. This mechanistic understanding is critical because it suggests that therapies that change the levels of this enzyme, but not those that block its function, might be good therapies 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 researchers are 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: None of at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

7. **Grant#:** 21B07 Cholinergic mechanisms underlying cognitive deficits during and following chemotherapy for breast cancer

Principal Investigator: Rex M. Philpot, PhD

Organization: University of South Florida

Summary: The effective doses of doxorubicin (DOX) and cyclophosphamide (CYP) used in humans were verified to effectively reduce tumor number and growth in the polyomavirus middle T antigen overexpression mouse model (MMTV-PyVT) mouse model. Disruptions in normal estrous cycling during chemotherapy but only temporary decreases in circulating estradiol levels. The study found that MMTV-PyVT tumor-bearing mice exhibited worse cognitive function compared to matched controls without tumors. This parallels findings in human literature, suggesting that the presence of tumors could contribute to cognitive impairments before chemotherapy treatment. Repeated administration of therapeutic doses of doxorubicin (DOX)+cytochromes P450 (CYP) resulted in a cognitive function deficit lasting at least 30 days beyond the treatment period, indicating that the chemotherapeutic agents themselves impair cognitive function. The study found that both tumors and chemotherapy affected the levels of macrophage-inflammatory protein 2 (MIP-2) and granulocyte colony-stimulating factor (G-CSF). MIP-2, associated with cognitive impairments, was elevated in tumor-bearing mice and affected by repeated chemotherapy. G-CSF, which plays a neuroprotective role, showed changes with chemotherapy exposure and tumor presence. Circulating interleukin-13 (IL-13), associated with cognitive improvement, was reduced by chemotherapy, potentially affecting cognitive function. Monocyte chemoattractant protein-1 (MCP1), linked with cognitive decline in Alzheimer's disease, showed changes with tumor presence and chemotherapy, contributing to persistent cognitive impairment. A preliminary analysis of choline acetyltransferase (ChAT) quantities and activity in different brain regions revealed intriguing findings. While ChAT levels in the central nervous system (CNS) did not differ by tumor presence or chemotherapy, ChAT activity was increased in the whole brain tissue of tumor bearing mice and mice administered chemotherapy. Interestingly, significant region-specific impairments in the frontal cortex and striatum ChAT

activity were observed after chemotherapy injection, especially in tumor-bearing mice. This suggests region specific chemotherapy-induced impairments in acetylcholine synthesis, potentially contributing to acute cognitive deficits and suggesting that drugs which can substitute for acetylcholine may provide a cognitive benefit. The researchers continue to breed and genotype MMTV-PyVT mice, tracking tumor development and measuring estrous cycling and tumor characteristics during behavioral assessments, chemotherapy and treatment.. To date there is no clear indication that either drug improves cognitive function in chemotherapy exposed mice, however the group sizes are too small to have sufficient statistical power. Preliminary evidence indicates that the nicotinic agonists PNU-282987 or RJR-2403 do not increase tumor growth or interfere with chemotherapy effectiveness, suggesting their potential as beneficial adjuvants for preserving cognitive function during chemotherapy if a cognitive benefit is observed.

Follow on Funding: None at the time of reporting.

Collaborations: None of 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, Inc.

Summary: 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, H. Lee Moffitt Cancer Center and Research Institute 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. Helpful input has been obtained from the Community Advisory Panel and members of the Scientific Advisory Board have also provided useful inputs. After delays related to COVID, recruitment was launched in clinics in Spring 2022. A total of 295 women were enrolled through June 30, 2023, and have completed study procedures with an average of five to six 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 of 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

Summary: The research team focused on determining if cell adhesion molecule 1 (CADM1)-T-lymphoma invasion and metastasis (1TIAM1) complex disrupts ubiquitin-editing enzyme A20 and cylindromatosis (CYLD) complexes after stimulation with either tumor necrosis factor alpha (TNFα) or interleukin-1 beta (IL-1β) to maintain chronic nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation in viral oncogenes expressing cells. The research team found that the loss of Tax1 Binding Protein 1 (TAX1BP1) and IκB kinase (IKK)α interaction and TAX1BP1 phosphorylation at Ser593, which is essential for the assembly of ubiquitin-editing A20 and CYLD complexes, in WT mouse embryonic fibroblast (MEFs) and Cadm1–/– MEFs reconstituted with wild-type Flag-CADM1 stimulated with TNFα, but not in viral oncogenes expressing Cadm1–/– or Cadm1–/– MEFs reconstituted with Flag-CADM1△ post synaptic density protein-binding motif (PDZ-BM) mutant stimulated with TNFα. In addition, the research team observed chronic K63-liked ubiquitination of receptor-interacting protein 1 (RIP1) in tumor necrosis factor receptors (TNFR) signaling and chronic nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα) phosphorylation and degradation and NF-κB activation in viral oncogenes expressing WT MEFs and Cadm1–/– MEFs reconstituted with wild-type Flag-CADM1 after stimulation with TNFα, but not in Cadm1–/– MEFs or Cadm1–/– MEFs reconstituted with Flag-CADM1△PDZ-BM mutant. The above discovery was made once (n=1), and it must be repeated at least three times (n=3) in order to reach valid scientific conclusions. Therefore, the research team repeated the experiments proposed in Aim 3.2 and validated the previously made findings. The preceding experiments were carried out on mouse fibroblasts and must be validated in primary human cells as well. Thus, the research team is continuing experiments using commercially available primary human cells, such as fibroblast and epithelial cells that are knocked down of CADM1 expression using 3'UTR lentiviral short hairpin ribonucleic acid (shRNA), which will only target endogenously expressing CADM1 and not exogenously expressing CADM1, and repeating the above experiments. Similarly, the research team will knock down TIAM1 and Rac1 expression using 3'UTR lentiviral shRNAs, and repeating the Aim 3.2 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#:** 21B11 Impact of the microenvironment on breast cancer genomic instability

Principal Investigator: Jerome Irianto, PhD

Organization: Florida State University

Summary: The development of patient-derived organoid culture in recent years creates an exciting opportunity for researchers to perform a wide range of in vitro studies on a model that closely recapitulates the tumor. One of the outstanding questions in cancer biology is the causes and consequences of genomic heterogeneity observed in the disease. However, to use organoids as a model to study genomic variations, researchers need to first understand the degree of genomic heterogeneity and its stability within organoids. The research project staff used single-cell whole-genome sequencing to investigate the genomic heterogeneity of two independent pancreatic cancer organoid lines, as well as their genomic stability with extended culture. In summary: the research staff showed that genomic heterogeneity can be observed in organoids through single cell DNA sequencing. Like in breast cancer, pancreatic cancer tumor is known for its high proportion of stroma which accounts for 90% of the tumor mass. The stroma is made up of extracellular matrix (ECM) and non-malignant cells such as inflammatory cells, cancer-associated fibroblasts (CAF), and lymphatic and blood vessels. Here, the research project staff decoupled the roles of the ECM on pancreatic ductal adenocarcinoma (PDAC) cell lines by culturing the cells on surfaces coated with different ECM proteins. The data showed that the primary tumor-derived cell lines have different morphology that depends on the ECM proteins on which they are cultured, while metastatic lesion-derived PDAC lines' morphology does not change with respect to the different ECM proteins. Similarly, ECM proteins also modulate the proliferation rate and the gemcitabine sensitivity of the primary tumor PDAC cell lines, but not for the metastatic PDAC lines. Lastly, transcriptomics analysis of the primary tumor PDAC cells cultured on different ECM proteins reveals the regulation of various pathways, such as cell cycle, cell adhesion molecules, and focal adhesion, including the regulation of several integrin genes that are essential for ECM recognition. In summary: the research staff showed that different cell types respond differently to ECM proteins.

Follow on Funding: None at the time of reporting.

Collaborations: Dr. Xian Fan's laboratory Florida State University (FSU) on the bioinformatics analysis in this project and Dr. Yue Julia Wang laboratory (FSU) on the single cell deoxyribonucleic acid sequencing (DNA-seq) and RNA-seq.

Journals: Usman, OH, Zhang, L, Xie, G, et al. Genomic heterogeneity in pancreatic cancer organoids and its stability with culture. *npj Genom. Med*. 7, 71 (2022). doi:10.1038/s41525-022-00342-9.

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, Inc.

Summary: University of Florida researchers are continuing curating CT images and clinical data on lung cancer patients treated with either immunotherapy, EGFR tyrosine kinase inhibitors (TKIs), and KRAS inhibitors. To date, University of Florida has curated images (baseline+/- pre-baseline) from nearly 2000 patients treated with immunotherapy and >600 patient treated with TKI (baseline+/- pre-baseline). Patient-level data collection efforts are still on-going. No results from the primary analyses have been generated yet; however, using a subset of data and images from the patients treated with immunotherapy the researchers published a multi-disciplinary study in Clinical Cancer Research (PMID: 37233986) showing that patient reported outcomes are predictive of early progression among patients treated with immunotherapy. A future R01 grant is in the planning stages to validate and expand this research

Follow on Funding: None at the time of reporting.

Collaborations: None of 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

Summary: This study aims to get a better understanding of the role and underlying mechanism of Human AlkB homolog H5 (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 deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequences by adding a tag (a methyl group), which can be recognized by other proteins in the cell. 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. During this period, University of Florida has made significant progress on this project. To further understand the function of ALKBH5 in leukemogenesis, research teams searched for the binding proteins of ALKBH5 in leukemia cells by liquid chromatography with tandem mass spectrometry (LC-MS-MS) analysis of the proteins pulled down by ALKBH5 co-immunoprecipitation (co-IP). In addition to RNA-binding protein 33 (RBM33), University of Florida has identified calpain 1 (CAPN1), eukaryotic translation elongation factor 2 (EEF2) and far upstream element binding protein 1 (FUBP1) as potential binding proteins of ALKBH5. University of Florida also found that Ca2+-activated cysteine protease, calpain-5 (hCAPN1) and eukaryotic translation elongation factor 2 (EEF2) play a critical role in regulating the proliferation of head neck cancer cells, suggesting that both genes have an oncogenic role in head neck cancer cells. Interestingly, hCAPN1 upregulation was correlated with a poor prognosis in AML patients. The University of Florida failed to detect a strong interaction between these three proteins and ALKBH5. To further determine the role of ALKBH5 in AML1-ETO-induced leukemogenesis, University of Florida inhibited ALKBH5 expression by ALKBH5- specific short hairpin ribonucleic acid (shRNA) in SKNO-1 cells carrying AML1-ETO fusion gene or expressed AML-ETO-9a fusion gene alone or together with N-RASG12D mutant in Alkbh5 knockout (KO) hematopoietic stem/progenitor cells. The results at the University of Florida is that ALKBH5

knockdown significantly inhibited SKNO-1 cells growth while Alkbh5 depletion also significantly inhibited the growth of primary mouse hematopoietic stem/progenitor cells. The University of Florida also found that Alkbh5 depletion enhances the growth of hematopoietic stem/progenitor cells expressing both AML ETO-9a and N-RASG12D mutant, raising the possibility that the function of Alkbh5 may be also dependent on cell context.

Follow on Funding: None at the time of reporting.

Collaborations: None of at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix D: William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program Fiscal Year 2022-2023 Active Grants Funded Fiscal Year 2019-2020

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
20B08	H. Lee Moffitt Cancer Center and Research Institute, Inc.	John M. Koomen, PhD	$253,555.00	11/30/23	No	No	Yes
20B13	University of Miami	Jaime Merchan, MD, MMSc	$636,610.00	11/30/23	No	No	Yes
20B15	University of Miami	Lluis Morey, PhD	$636,610.00	11/30/23	Yes	No	Yes
20B16	University of Miami	Paulo S. Pinheiro, PhD	$750,000.00	11/30/23	No	No	Yes

1. **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, Inc.

Summary: The research team is focused on discovery proteomics to elucidate the differences between metastatic lesions in different organs, the research completed bioinformatics analysis of the samples collected from non-small cell lung cancer patients. The team has been able to acquire data to assess the proteomes of 46 tumors and metastatic lesions from small cell lung cancer patients, and data analysis is ongoing. The goal is to examine differences between tumors and metastases within the same patient first and then examine common features of metastases in specific organs (e.g. liver and lymph node). The research team was able to acquire additional discovery proteomics datasets for a clinical trial: “Combination Immunotherapy-Ipilimumab-Nivolumab-Dendritic Cell p53 Vac - Patients With Small Cell Lung Cancer (NCT03406715).” In addition, the research team were also able to analyze samples from a retrospective analysis of Moffitt’s small cell lunch cancer guide (SCLC) patients treated with Lurbinectedin to try to link differences in the pre-treatment tumor proteomes to patient response. These analyses are being integrated with the other proteomics data acquired from the same SCLC patients in the rapid tissue donation program. For targeted proteomics, the research team wanted to improve the ability to communicate complicated targeted proteomics data to clinicians to enable easier access to data from patient samples and gain traction for this technology to be applied in the clinic. The study’s co-investigator developed a software application called Physician-Interpretable Phenotypic Evaluation in R (PIPER), which enables examination of targeted proteomics data to provide information for an individual patient and biomarker to mapping pathway-level information for multiple protein biomarkers. Different graphing capabilities have been added to help visualize the data both by amount of protein and by the rank for that protein’s expression in the patient cohort (quantile-based display) to use these biomarker measurements to complement genomics approaches for the selection of therapy for lung cancer patients. This application has been made available to the community through GitHub, the example data have been made publicly available through ProteomeXchange and Panorama Public, and a paper published in the Journal of Proteome Research. For targeted proteomics, the research team has also started assay development to enable quantification of therapeutic monoclonal antibodies (Ipilumumab and Nivolumab used for

the clinical trial described above) to understand tissue penetration of these therapies in lung tumors and their metastases.

Follow on Funding: None at the time of reporting.

Collaborations: Misty Shields, MD, completed a fellowship and moved from Moffitt to a faculty position at Indiana University (Simon Cancer Center); researchers continue to collaborate for the analysis of the samples from the retrospective study of lurbinectedin treated patients. No trainees have been involved in the research during this period.

Journals: Putty Reddy S, Alontaga AY, Welsh EA, et al. Deciphering Phenotypes from Protein Biomarkers for Translational Research with PIPER. *J Proteome Res*. 2023;22(6):2055-2066. doi:10.1021/acs.jproteome.3c00137.

Patents: None at the time of reporting.

2. **Grant #:** 20B13 Tumor and Strama/ Targeted Oncolytic Virus based Biotherapies for Colorectal Cancer

Principal Investigator: Jaime Merchan, MD, MMSc

Organization: University of Miami

Summary: 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 University of Miami had developed novel measles virus vectors, which are able to target tumor stroma, the University of Miami also has targeted vectors, allowing the research team to characterize the effects in syngeneic, immunocompetent colorectal cancer models, in addition to human CRC models. The University's 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 University of Miami has completed Aim 1, and has made significant advances in Aim 2. In Aim 1, the University of Miami 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 University's in vivo studies, where the University of Miami confirmed potent in vivo antitumor activity of minnelide in two different human colon cancer models (HT-29 and HCT-116), as the University of Miami ll as in murine colon cancer (CT-26). Moreover, the research team 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 the University of Miami reobserved 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 University of Miami 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, the University of Miami 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. The University of Miami has presented the results of the above studies in international scientific meetings.

Follow on Funding: None at the time of reporting.

Collaborations: None of at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Lluis Morey, PhD

Organization: University of Miami

Summary: Due to regulations that the University of Miami (UM) put in place to mitigate the COVID outbreak, the lab was operating at 25% of its capacity. Nevertheless, the research team were currently performing RING1-B pull-downs after 45 minutes of estradiol (E2) stimulation in T47D and MCF-7 cells. Researchers characterized the T47D-ring chromosome 18 (RING18)-activation-induced cytidine deaminase (AID)-green fluorescent protein (GFP) cells and are currently performing the first set of experiments. The research staff have also decided to use short hairpin ribonucleic acid (shRNA)-RING1 B cells as a first approach to determine the role of RING1B in chromatin architecture. The reason is because the research staff were still characterizing the T47D-RING1B-AID-GFP cells. Moreover, shorthairpin ribonucleic acid (shRNA)-based experiments are very useful to establish a preliminary indication. As proposed, researchers performed assay for transposase-accessible chromatin (ATAC) experiments. Researchers have already performed chromatin immunoprecipitation exonuclease (ChIP-exo) of grainyhead-like transcription factor 2 (GRHL2) before and after 45 minutes of E2. Researchers also performed chromatin immunoprecipitation (ChIP)-quantitative polymerase chain reaction (qPCR) of GRHL2 in control and RING1B knock down (KD) cells. Research staff confirmed co-occupancy of GRHL2 with RING1B and Forkhead box protein A1 (FOXA1) after E2 administration. The research staff also started experiments for Aim 2.1 and 2.3 by performing ChIP-seq of FOXA1, RING1B and ER in wild type of ER mutant cells (estrogen receptor alpha (ERα) Y537S and ERα D538G). The research staff are currently analyzing the results. Research staff also performed ribonucleic acid (RNA)-seq experiments in RING1B depleted cells that are resistant to endocrine therapy like ERα Y537S and ERα D538G cells, but also in a newly developed cell-based model system that mimics resistance to aromatase inhibitors (a common endocrine treatment). Overall, research staff estimates that six months is still needed to finalize the in vivo experiments. The reason is because the beginning of the grant was impacted by COVID and UM regulations regarding return to work. Moreover, the researchers had problems in hiring a bioinformatician, who just started in February 2023. Nevertheless, the researchers published two high impact factor papers, with another one under review and are preparing two more.

Follow on Funding: Morey, L, PI; Mechanisms of RING1B and PRC1 complexes in transcriptional activation. NIH-NIGMS R01 09/01/2022-08/31/2026. Total Amount Awarded: $1,716,885. Award Notice Date: 12/16/2022. CFDA Code: 859.

Morey, L, PI; Altering the chromostasis and genome stability by modulating histone methylation. NIH-NIGMS R01 09/02/2022-05/30/2026. Total Amount Awarded: $425,068. Award Notice Date: 06/13/2023. CFDA Code: 859.

Collaborations: Department of Computer Science, University of Miami, Department of Biochemistry and Molecular Biology, University of Miami, Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY, Department of Medicine, Division of Hematology, University of Miami, Department of Molecular Medicine, Mays Cancer Center, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, Division of Surgical Oncology, Department of Surgery, University of Miami, Cancer Research Program, IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain, Department of Experimental Oncology, European Institute of Oncology (IEO), IRCCS, Milan, Italy.,Department of Biosciences, University of Milan, Milan, Italy.,Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain, Department of Biomolecular Chemistry, School of Medicine and Public Health, University of Wisconsin, Madison, WI 53706, USA.

Journals: Garcia-Martinez, L, Zhang, Y, Nakata, Y, et al. Epigenetic mechanisms in breast cancer therapy and resistance. *Nat Commun 12*, 1786 (2021). Doi:10.1038/s41467-021-22024-3.

Zhang Y, Liu T, Yuan F, et al. The Polycomb protein RING1B enables estrogen-mediated gene expression by promoting enhancer-promoter interaction and R-loop formation. Nucleic Acids Res. 2021;49(17):9768-9782. doi:10.1093/nar/gkab723

Garcia-Martinez L, Adams AM, Chan HL, et al. Endocrine resistance and breast cancer plasticity are controlled by CoREST. *Nat Struct Mol Biol*. 2022;29(11):1122-1135. doi:10.1038/s41594-022-00856-x

Patents: None at the time of reporting.

4. **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

Summary: The aims of the current research project are to identify critical points in disparities in risk (incidence) and survival for two highly fatal cancers (lung and liver) among the different racially detailed populations of Florida (e.g., Cubans, Puerto Ricans, Afro-Caribbeans, Whites. African Americans etc.). The disparities in relation to etiology for lung (e.g., smoking) and liver (e.g., liver hepatitis) among these racial-ethnic populations are unknown in Florida (and elsewhere); therefore, hindering efforts to properly control and prevent these malignancies. The current project has completed its third year and after delays due to dataset authorizations, all data sets have been received and necessary abstractions were completed in December 2022. The following is a status update of the respective aims of the grant: Aim 1: The research team has been able to assess patterns in lung cancer, as a whole, and in never-smokers. The researchers were able to produce, for the first time in the US and, to the knowledge of the researchers, the first time in the world, population-based (cancer registry-based) rates of lung cancer in never smokers, which is an important disease, especially in women and ethnic minorities. Furthermore, lung cancer incidence rates in Florida reveal nearly three-fold difference within aggregate racial-ethnic groups such as among non-Hispanic Blacks (US-born Black males versus Caribbean-born Black males) and among Hispanics (Cuban males versus

Central American males), revealing disparities normally obscured by large grouping. Kidney cancer (KC) survival was also assessed with focus on smoking status finding that smoking independently contributes to poorer survival, across all KC stages. For liver cancer, researchers have also produced, for the first time in the US and, to the knowledge of the researchers, the world, population-based (cancer registry-based) rates by etiology, race, and ethnicity, which have revealed distinct patterns by etiology for different racial-ethnic groups in Florida. Furthermore, researchers have estimated liver cancer incidence by detailed race-ethnicity subgroup which is novel on multiple fronts due to the diversity of Florida and availability of robust racial-ethnic and nativity data. These findings suggests that prevention, screening, and clinical surveillance may be better tailored according to race-ethnicity. Aim 3: To increase liver cancer screening and prevention via awareness of undiagnosed chronic hepatitis C and B. The researchers have partnered with the Health Choice Network (HCN), a cohesive healthcare organization in Florida, in the development of a continued medical education program targeting medical professionals working with higher risk populations of Florida.

Follow on Funding: None at the time of reporting.

Collaborations: Dr. Pinheiro continues to work as a Consultant on Cancer in Hispanics Surveillance Systems with the National Cancer Institute's (NCI's) Surveillance, Epidemiology, and End Results (SEER) Program. Ms. Kamaria Jacobs, doctoral candidate at Florida A&M University (FAMU)- Institute of Public Health, College of Pharmacy and Pharmaceutical Sciences (Tallahassee, FL) continues to work on a project related to Aim 1.2. Other collaborations for different manuscripts in Aim 1.1. include Dr. Scarlett Gomez from the University of California San Francisco, Dr. Karen Callahan from the University of Nevada Las Vegas. For Aim 2, Dr. Pinheiro has collaborated with Dr. Wendy Setiawan's team from the University of Southern California on a study on hepatocellular carcinoma in Hispanics. In addition, Dr. Pinheiro has collaborated with Dr. Timothy Bungum from the University of Nevada Las Vegas, Dr. Robert Wong of Stanford University, and Dr. Kathryn McGlynn from the National Institutes of Health (NIH). For Aim 3, further collaborations and meetings have occurred with Dr. Patricia Jones of University of Miami School of Medicine, Dr. Robert Wong of Stanford University, and stakeholders at the Health Choice Network (HCN), a cohesive healthcare organization consisting of 25 health centers and 325 care delivery sites in Florida. Leveraging newly performed research and findings from Aim 1.1. and a secondary data analysis of HCN data for HBV/HCV screening of the local Florida patient population, Dr. Pinheiro, Dr. Jones, and HCN leaders are generating an educational program specific to liver cancer screening and prevention via awareness of undiagnosed chronic hepatitis C and B, targeting medical professionals working with in higher risk populations of Florida.

Journals: Pinheiro PS, et al. Lung cancer in never smokers: Distinct population-based patterns by age, sex, and race/ethnicity. *Lung Cancer*. 2022 Dec;174:50-56. doi:10.1016/j.lungcan.2022.10.009.

Liu Q, et al. 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. doi:10.1200/JCO.2022.40.

Cranford HM, Koru-Sengul T, Lopes G, Pinheiro PS. Lung Cancer Incidence by Detailed Race–Ethnicity. *Cancers*. 2023; 15(7):2164. doi:10.3390/cancers15072164.

Baral A, Cranford HM, Sharma J, Pinheiro PS. The prognostic role of cigarette smoking in Kidney Cancer Survival. *Cancer Med*. 2023, doi:10.1002/cam4.6104.

Liu Q, Koru-Sengul T, Lopes G, Pinheiro PS. The association of race and curative-intent treatment with mortality outcomes among early-stage NSCLC. *Journal of Clinical Oncology*. 2023 June 1, Vol 41 (16_suppl), e20563-e20563. doi:10.1200/JCO.2023.

Pinheiro PS, Cranford HM, Liu Q, Jones PD. Epidemiology of cholangiocarcinoma in Florida, and differences in risk factors between intra- and extra-hepatic cholangiocarcinoma. *Journal of Clinical Oncology*. 2023 June 1, Vol 41 (16_suppl), e16329-e16329. doi:10.1200/JCO.2023.41.

Patents: None at the time of reporting.

Appendix E: William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program Fiscal Year 2022-2023 Active Grants Funded Fiscal Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
9BC07	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Gina M. DeNicola, PhD	$1,335,000.00	5/31/24	Yes	No	Yes
9BC08	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Nelli Bejanyan, MD	$1,335,000.00	3/31/24	Yes	No	Yes

1. **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, Inc.

Summary: Lung cancer is the leading cause of cancer-related death. Mutations in the NF-E2 p45-related factor 2 (NRF2)/Kelch-like ECH-associated protein 1 (KEAP1) circuit are among the most common mutations in lung cancer, are suggested to cause chemo/radio resistance, and are enriched in tumors that fail to respond to targeted therapy. Research project staff are evaluating new therapeutics specifically designed to target NRF2/KEAP1 mutant tumors and determine whether these mutations are broadly associated with responses to all standard treatments, which may lead to better precision medicine. Prior research described a focused genetic screen to identify NRF2-regulated genes that mediate resistance of lung cancer cells to reactive oxygen species (free radicals). H. Lee Moffitt Cancer Center and Research Institute, Inc. (Moffitt) published the results of one of these targets, called Superoxide dismutase 2 (SOD2). In this reporting period, the researchers have focused on another target glutathione-disulfide reductase (GSR), which researchers find mediates the resistance of mitochondria to free radicals. The research team also performed another genetic screen for direct regulators of NRF2 itself and identified a role for the mitochondrial electron transport chain in NRF2 stability. Finally, in parallel studies, the research team has also found that cancer cells with NRF2/KEAP1 mutations are sensitive to starvation of the metal copper because research has been done on levels of copper. Studies also suggest that the mutations are sensitive to copper overload as Research ll. Researchers are currently performing studies to understand why copper levels are higher and how researchers can leverage this information for therapy. Finally, the researchers are evaluating the effect of KEAP1 and NRF2 mutation status on patients' response to chemotherapy, radiation therapy and immunotherapy. In a prior reporting period, the research team completed the analysis of KEAP1/NRF2 mutation status with radiation response. The Center's current work is focused on the chemotherapy response. Cohorts for the analysis of chemotherapy response the research team reassembled 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.

Follow on Funding: Chang, J, PI; Molecular control of bone development and inflammation by FBXO11. NIH/NCI K99/R00 09/01/2022-08/31/2024. Total Funds Awarded: $362,188. Award Notice Date: 04/13/2023. CFDA Code: 121.

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. Dr. Williams is analyzing the association between KEAP1/NRF2 mutations and tumor response to radiation. Following the death of colleague collaborator David Boothman, who provided b-lapachone for these studies and was going to provide the more potent compound IB-DNQ, researchers have established a collaboration with Dr. Paul Hergenrother, Department of Chemistry, University of Illinois at Urbana-Champaign to obtain IB-DNQ

Journals: Pezacki AT, Matier CD, Gu X, et al. Oxidation state-specific fluorescent copper sensors reveal oncogene-driven redox changes that regulate labile copper(II) pools. *Proceedings of the National Academy of Sciences of the United States of America*. 2022;119(43) doi:10.1073/pnas.2202736119.

Patents: None at the time of reporting.

2. **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, Inc.

Summary: This study's purpose is to study the safety and potential effectiveness of expanded donor-derived gamma delta (γδ) T cells for treatment of adverse genetic risk acute myeloid leukemia after stem cell transplantation in phase one trial. Stem cell transplantation (SCT) is the only curative treatment for most adult patients with acute myeloid leukemia (AML), which is the most common acute leukemia in adults. Leukemia recurrence, however, remains the leading cause of mortality after SCT. In most recent years, promising early results are reported with use of γδ T cell subsets for treatment of various cancers. However, the number of circulating γδ T cells in a blood is very low. Thus, stimuli to expand the number of circulating γδ T cells in a blood is needed for effective use of these cells as anticancer therapy. Researchers aimed to use healthy donor derived γδ T cell immunotherapy to reduce the leukemia recurrence risk after SCT in patients with adverse genetic risk AML. The research team used artificial antigen presenting cells (AAPC) that support T cell activation and expansion in laboratory conditions and achieved more than 600-fold expansion of healthy donor blood γδ T cells. This trial is evaluating the safety of donor γδ T cells in three dose levels. The enrollment of patients in dose level one was completed and researchers analyzed the results. None of the treated patients in dose level one experienced any dose limiting toxicity. It was observed no cases of cytokine release syndrome or neurological toxicities in these patients. No cases of leukemia recurrence were observed despite all treated patients being at high risk of recurrence due to having an adverse genetic risk AML, being elderly and receiving reduced-intensity conditioning (RIC) stem cell transplantation (SCT). Thus, enrollment of patients in dose level 2 proceeded. The research team is now preparing an abstract to report this promising experience at the American Society of Hematology annual conference in December 2023. Impact to Floridians: AML is a disease of elderly with average age at diagnosis of 68 years. Since at least 25% of Florida population is elderly (over 60 years old) AML, particularly adverse genetic risk, is more common in this age group. In addition, elderly patients are at high risk of leukemia recurrence after SCT as patients would only be a candidate for RIC transplant. Thus, if donor γδ T cell immunotherapy is proven safe and effective in controlling leukemia recurrence after SCT, this can increase the cure from adverse risk AML and benefit Floridians.

Follow on Funding: Bejanyan, N, PI. Engineering immune cells to fight leukemia. Merit Society Foundation 02/18/2022 Total Funds Awarded: $20,000.00.

Collaborations: Initiated additional collaborations with Laboratory Scientists at Moffitt Immunology Department.

Journals: Boucher JC, Yu B, Li G, et al. Large Scale Ex Vivo Expansion of γδ T cells Using Artificial Antigen-presenting Cells. *J Immunother*. 2023;46(1):5-13. doi:10.1097/CJI.0000000000000445.

Patents: None at the time of reporting.

Appendix F: William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program Fiscal Year 2022-2023 Closed Grants Funded Fiscal Year 2021-2022

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
22B11	University of Florida	Guangrong Zheng, PhD	$100,000.00	9/30/22	No	No	No

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

Principal Investigator: Guangrong Zheng, PhD

Organization: University of Florida

Summary: 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. Immunotherapy such as anti-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. 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. The 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.

Appendix G: William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program Fiscal Year 2022-2023 Closed Grants Funded Fiscal Year 2019-2020

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
20B01	All Children's Research Institute	Masanobu Komatsu, PhD	$636,611.00	4/30/23	Yes	No	Yes
20B03	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Shain Kenneth, MD, PhD	$636,610.00	5/31/23	No	No	Yes
20B04	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Paulo C. Rodriguez, PhD	$636,610.00	5/31/23	No	No	Yes
20B06	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Andriy Marusyk, PhD	$636,610.00	4/30/23	Yes	No	Yes
20B10	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Nicholas J. Lawrence, PhD	$636,610.00	11/30/22	No	Yes	Yes
20B11	Relinquished						
20B12	University of Miami	Sabita Roy	$636,610.00	5/31/23	No	No	No
20B14	Relinquished						
20B17	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Chen Jiandong, PhD	$636,610.00	6/30/23	No	No	No

1. **Grant#:** 20B01 Reprograming tumor immune landscape by high endothelial venule formation

Principal Investigator: Masanobu Komatsu, PhD

Organization: All Children's Research Institute

Summary: The network of blood vessels grown in tumors (tumor vasculature) shapes the immune landscape through the recruitment of immune cells such as lymphocytes. As gateways for the lymphocyte entry to tumors, a type of specialized blood vessels, high endothelial venules (HEVs), would enable abundant lymphocyte infiltration and create an inflamed microenvironment that is favorable for immunotherapy. Through investigating HEVs and non-HEV blood vessels in human malignant tumors, this project has discovered a potentially clinically applicable strategy to induce the formation of tertiary lymphoid structures (TLS) in these tumors. This means that researchers now know the way to create immunostimulatory tumor microenvironment to enhance cancer immunotherapy as well as conventional chemotherapy. This finding was achieved by comparing the endothelium of the tumor vasculature between immune hot and immune cold tumors. Such a study led research staff to test the agonists (stimulating drugs) of two innate immunity pathways, lymphotoxin beta receptor (LTβR) agonist and stimulator of interferon genes (STING) agonist. The combination treatment with the two agonists induced TLS formation and achieved long-term prevention of tumor recurrence and metastasis. The STING agonist is already in clinical trial, and the LTβR agonist can be produced for clinical use. This finding therefore offers a potential breakthrough in cancer patient care. The method to induce TLS in mouse tumors by STING and LTβR activation opened up an unprecedented opportunity to examine the precise role of TLS in anti-tumor immunity. Researchers can now take advantage of this model to determine the mechanism of TLS formation, the sequence of events, immune activating activities, and its contribution to enhancing immune response to cancer.

Follow on Funding: Komatsu, M. (2022). Role of intratumoral high endothelial venules in tumor immunity. NIH-NCI R01 04/01/2022–03/31/2027. Awarded 03/20/2023.

Collaborations: Zhong, C, PhD, Assistant Professor, Department of Electrical Engineering and Computer Science, University of Kansas. Dr. Zhong and the research group process ribonucleic acid (RNA) sequencing data from bulk tumor RNA and single-cell analysis for these projects.

Journals: Sawada J, Hiraoka N, Qi R, et al. Molecular signature of tumor-associated high endothelial venules that can predict breast cancer survival. *Cancer Immunology Research*. 2022; 10(4):468-481. doi:10.1158/2326-6066.CIR-21-0369.

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, Inc.

Summary: Research staff have now successfully developed an alternative high-throughput screen to examine the cellular activity of potential Type IIA topoisomerase (TOP2A) nuclear export signals (NES) specific compounds. This involved the use of the Ex vivo Mathematical Myeloma Advisor (EMMA) system. Research staff will use the combination activity between Doxorubicin and identified compounds in a 1536 well plate format using the Mosquito™ liquid plate handler. With one isogenic cell line and five doses of each drug or the combination, this platform will facilitate testing of about 100 compounds (or about 50 with two multiple myeloma (MM) (or other cancer model cell lines)). Researchers plan to compare these two high-through put methods on multiple levels—contrasting phenotype (increased death) versus biologic activity inhibition of TOP2A-Exportin 1 (XPO1) complexes (or other XPO1 targets) as well as screen compounds identified from Life Chemical Library and Chembridge Library. Researchers are in the process of updating the nanoelectro spray ionization (NESi) screening manuscript and anticipate resubmission. In conclusion, researchers have successfully developed and utilized a screening tool for testing putative TOP2A NESi. The research team identified a lead compound NSC9138 and demonstrated that it functioned in a TOP2A specific manner.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Nemudraia A, Nemudryi A, Buyukyoruk M, et al. Sequence-specific capture and concentration of viral RNA by type III CRISPR system enhances diagnostic. Preprint. *Res Sq*. 2022;rs.3.rs-1466718. 04/19/2022. doi:10.21203/rs.3.rs-1466718/v1.

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, Inc.

Summary: The immunosuppressive tumor microenvironment (TME) orchestrated by epithelial tumors, such as melanoma and lung cancer, represents a challenge for the successful development of curative cellular immunotherapies, including chimeric antigen receptor (CAR)-T cells. Unfortunately, there are no current therapeutic strategies to fully overcome the immunosuppressive actions of the TME on transferred therapeutic T cells in cancer hosts. These results show that overexpression of Notch1 intracellular active domain in T cells, or expansion of T cells in the presence of high affinity variants of Notch ligand Delta-like 4 (DLL4), boost their anti-tumor effects after adoptive transfer, which correlated with an increased mitochondrial fitness and T cell expansion. Based on the strong preliminary findings, researchers hypothesize that the activation of the Notch pathway makes adoptively transferred T cells highly efficient at eliminating tumors. Obtained results during the current period can be summarized as follows: These results set the foundation for new therapeutic strategies to rescue the activity of stressed T cells in tumors and also enable the development of CAR-T cells with a higher capacity to eliminate solid malignancies by targeting mitochondrial related processes. The research staff continued with a study aiming to understand the anti-tumor effector responses of follicle-stimulating hormone-chimeric endocrine receptor (FSH-CER) and endogenous T-cell receptor-silenced and affinity-enhanced (NY-ESO-TCR) transduced T cells having heightened Notch signaling induced after expansion in the presence of artificial antigen-presenting cells (aAPCs) expressing high affinity DLL4 ligand (DLL4.v3). Researchers found that FSH-CER T cells expanded in the presence of aAPCs expressing high affinity DLL4 induced significant higher anti-tumor effector cytokines interferon-gamma (IFN-γ) and granzyme B, which correlated with elevated Notch signaling. This is very impactful as it could set a new approach to be used in therapeutic T cells. To evaluate the role of DLL4.v3 conditioned T cells in vivo, research staff next performed adoptive transfer of TCR-transduced T cells into immunocompromised NSG mice bearing NY-ESO-1 expressing human tumors. Researchers found that DLL4.v3 conditioned T cells were able to restrict the tumor growth more efficiently than mock conditioned T cells. Additionally, inhibition of Notch prevented their cytotoxic activity. Thus, the anti-tumor actions induced high affinity DLL4 were entirely mediated by Notch signaling in T cells. In the last cycle of funding, researchers found that DLL4.v3-related induction of Notch boosted effects on effector and memory T cells, which correlated with higher anti-tumor potential. In summary, this study successfully developed a new strategy to increase the activity of therapeutic T cells for the benefit of cancer patients in the state of Florida.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Mandula JK, Rodriguez PC. Tumor-directed dysregulation of erythroid progenitors drives immunosuppressive myeloid cells. *Cancer Cell*. 2022;40(6):597-599. doi:10.1016/j.ccell.2022.04.017.

Mandula JK, Chang S, Mohamed E, et al. Ablation of the endoplasmic reticulum stress kinase PERK induces paraptosis and type I interferon to promote anti-tumor T cell responses. *Cancer Cell*. 2022;40(10):1145-1160.e9. doi:10.1016/j.ccell.2022.08.016.

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, Inc.

Summary: The progress within the project is not linear, as most of the efforts were directed towards testing multiple ideas and carving the path forward, followed by multiple rounds of refinement. Specific details could be found in the quarterly reports submitted through the course of the study. The following description outlines the overall project. Following comparison of several digital segmentation platforms, a cloud-based Aiforia platform was chosen based on the balanced consideration of ease of use, quality of segmentation, convenience, availability and responsiveness of technical support and price. The segmentation protocol had to be optimized for each of the individual model, which entailed using the training imaging to manually annotate different regions of interest (stroma, necrotic area, marker positive tumor cells, marker negative tumor cells), feeding this into artificial intelligence (A)I-based algorithm. Then, testing the Al based segmentation on a different sample or different region of the original sample. For most of the models, researchers needed four to five rounds of segmentation before the research staff were able to obtain satisfactory segmentation. Based on these studies, researchers have picked distance to nearest stroma and radial distribution function for most of the histological analyses of experimental and clinical samples, while applying additional functions when they are called for. While it is obvious that treatment leads to a dramatic reduction in the fraction of bromodeoxyuridine (BrdU)+ cells, and, upon treatment, positive cells are biased to peri-stromal locations, it is not obvious whether there is any bias in the distribution at the baseline. Nor it is obvious how to measure the magnitude and distance of the stromal bias under therapy. The commonly used and the most intuitive metrics of distance to nearest stroma is sufficient to discriminate between the presence or absence of bias. Resource Description Framework (RDF) covers this gap, producing quantitative assessment of both magnitude and distance of the stromal bias effect.

Follow on Funding: Marusyk, S, PI. NIH-NCI-PSON. Extending experimental evolutionary game theory in cancer in vivo to enable clinical translation: integrating spatiotemporal dynamics using mathematical modeling. 07/01/2023-06/31/2028. Application Number: 1U01CA280829-01. Total Amount Awarded: $2,076,612.00.

Marusyk, S, PI. Moffit Cancer Center: Team Science Award. Impact of stromal sheltering on acquisition of resistance to targeted therapies in lung cancers. 01/01/2022-12/31/2022. Total Amount Awarded: $150,000.00.

Collaborations: None at the time of reporting.

Journals: Kimmel GJ, Beck RJ, Yu X, et al. Intra-tumor heterogeneity, turnover rate and karyotype space shape susceptibility to missegregation-induced extinction. PLOS Computational Biology 19(1): e1010815. (2023) doi:10.1371/journal.pcbi.1010815.

Ferrall-Fairbanks MC, Dhawan A, Johnson B, et al. Progenitor Hierarchy of Chronic Myelomonocytic Leukemia Identifies Inflammatory Monocytic-Biased Trajectory Linked to Worse Outcomes. Blood Cancer Discov. 2022;3(6):536-553. doi:10.1158/2643-3230.BCD-21-0217.

Ferrall-Fairbanks MC, Chakiryan NH, Chobrutskiy BI, et al. Quantification of T- and B-cell Immune Receptor Distribution Diversity Characterizes Immune Cell Infiltration and Lymphocyte Heterogeneity in Clear Cell Renal Cell Carcinoma. Cancer Res. 2022;82(5):929-942. doi:10.1158/0008-5472.CAN-21-1747.

Andor N, Altrock PM, Jain N, Gomes AP. Tipping Cancer Cells Over the Edge: The Context-Dependent Cost of High Ploidy. Cancer Res. 2022;82(5):741-748. doi:10.1158/0008-5472.CAN-21-2794.

Johnson B, Altrock PM, Kimmel GJ. Two-dimensional adaptive dynamics of evolutionary public goods games: finite-size effects on fixation probability and branching time. R Soc Open Sci. 2021;8(5):210182. Published 2021 May 26. doi:10.1098/rsos.210182.

Miroshnychenko D, Miti T, Kumar P, et al. Paracrine enhancement of tumor cell proliferation provides indirect stroma-mediated chemoresistance via acceleration of tumor recovery between chemotherapy cycles. Preprint. bioRxiv. 2023;2023.02.07.527543. Published 2023 Jul 27. doi:10.1101/2023.02.07.527543.

Patents: None at the time of reporting.

5. **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, Inc.

Summary: The research team have designed and synthesized a series of new janus kinase 2 (JAK2) inhibitors that will serve as new drugs to treat patients with myeloproliferative neoplasms (MPNs). These have been characterized for the target engagement with JAK2 and preliminary data shows that they potently kill JAK2-driven cancer cells (that are relevant to MPNs) in vitro. The research staff have developed and reported a new method to purify and crystallize the kinase domain of the JAK2 protein and solved the X-ray crystal structures of several clinically approved JAK2 inhibitors and new inhibitors developed in the current project. This has revealed the key molecular interactions between the inhibitors and the target JAK2 protein, which have been exploited in the design of more potent compounds in the study. Most importantly the structures revealed the most appropriate linking strategy for the design of the heterobifunctional compounds (including proteolysis targeting chimeras (PROTACs)) in the study. The development of heterobifunctional compounds was a successful part of the project, which demonstrated that JAK2 PROTACs can be used to promote degradation of JAK2 in MPN cell models. Focus has been on developing methods for Inhibitor and PROTAC synthesis and assay and production of JAK2 for crystallographic and biophysical analyses, and biological assessment of anticancer properties and mode of action studies.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Davis RR, Li B, Yun SY, et al. Structural Insights into JAK2 Inhibition by Ruxolitinib, Fedratinib, and Derivatives Thereof. J Med Chem. 2021;64(4):2228-2241. doi:10.1021/acs.jmedchem.0c01952.

Patents: Lawrence, N, Lawrence, H, Schonbrunn, E, Reuther, G, Inventors. H. Lee Moffitt Cancer Center and Research Institute, Inc., assignee. INHIBITORS AND DEGRADERS OF JANUS KINASE 2. PCT/US2022/012772. January 18, 2022.

Schonbrunn, E, Lawrence, NJ, Lawrence, HR, Inventors. BRD4-kinase inhibitors as cancer therapeutics, US10,738,016, August 11, 2020.

Schonbrunn, E, Lawrence, NJ, Lawrence, HR, Inventors. H. Lee Moffitt Cancer Center and Research Institute, Inc., assignee. Potent dual BRD4-kinase inhibitors as cancer therapeutics, US patent application No. 63/178,363 (Moffitt ID No. 21MA004PR2) filed 04/22/2021.

Schonbrunn, E, Lawrence, NJ, Lawrence, HR, Inventors. STRUCTURAL INSIGHTS INTO JAK2 INHIBITION BY RUXOLITINIB, FEDRATINIB, AND DERIVATIVES THEREOF, PCT Serial Number PCT/US2022/012772. filed 01/18/2022.

The new JAK2 inhibitors are currently under advanced discussion with a biotech company, to fund a sponsored research agreement to further develop the inhibitors with a view to selecting a clinical safety candidate for IND-guided studies. Licensing agreements based on the patent filings issued and submitted during the project study are being pursued by the Moffitt Cancer Center Innovation Office.

6. **Grant#:** 20B12 Targeting the gut microbiome to improve cancer pain management by opioids

Principal Investigator: Sabita Roy

Organization: University of Miami

Summary: 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 your senses, causing you to think unclearly, trouble holding a job or maintaining normal social relationships. Therefore, controlling pain is an essential part of cancer treatment. The most common analgesics prescribed for moderate to severe cancer pain are OPIOIDS. Opioids use has been shown to induce microbial dysbiosis and systemic inflammation. University Of Miami in previous progress reports reported the development of a model for metastatic cancer pain. The current progress report determined that antibiotics treatment prior to cancer cell injection rapidly depleted essential commensal bacteria resulting in decrease in alpha diversity. University Of Miami previously showed that antibiotic treated mice displayed greater pain sensitivity. From these readouts the Unviversity of Mimai conclude that commensal bacteria are essential to protect against cancer associated pain. Bioinformatics analyses were performed as described. Demultiplexed sequence reads were clustered into amplicon sequence variants (ASVs) with the deficiency of adenosine deaminase 2 (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 sample were 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 time diversity core-metrics-phylogenetic script with sampling.

Follow on Funding: None at the time of reporting.

Collaborations: None of at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

7. **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, Inc.

Summary: Experiments in the final year of this grant covered several areas that aim to understand the mechanism of action of the validated p53 inhibitor NSC194598, use NSC194598 as a ligand to promote p53 degradation, and search for new regulators of p53 activity through high throughput drug screen. Recently proteolysis targeting chimeras (PROTAC) compounds emerge as potential therapeutics for targeting previously non-druggable proteins. The p53 inhibitor NSC194598 identified previously is a potential ligand for specific targeting of p53 for ubiquitination and degradation. The research team has designed and synthesized a series of chimeric compounds containing NSC194598 derivative linked to ubiquitin E3 Cereblon (CRBN) binding ligand pomalidomide. The new PROTAC compounds retained the ability to interact with p53 and CRBN, but did not show significant activity in p53 degradation. Therefore, the design preserved the binding activities of both ligands, but further optimization such as testing different linker length, linker chemistry, or recruiting different E3 ligases will be needed to obtain p53 degradation activity. Mechanistic analysis of p53 inhibition by NSC194598 identified several effects of the compound on p53: loss of binding to a wild type p53-specific antibody, formation of p53 aggregate in cells and in test tube and altered chemical crosslinking products. These effects suggest the compound binds to p53 and induces significant conformational change in the deoxyribonucleic acis (DNA) binding domain, resulting in loss of DNA binding affinity. Molecular docking suggests NSC194598 may bind to a cavity formed in the dimer of DNA binding domain, consistent with absence of deep ligand binding pocket in monomeric DNA binding domain of p53. Improved assays are being considered to map NSC194598 binding site on purified p53. The proposed high throughput screen of ~240,000 compounds for regulators of p53 transcription activity using reporter cell lines. The research team have identified five confirmed candidates that showed the ability to increase or inhibit p53 activity in cells. Further analysis of these hit compounds may result in new regulators of p53 activity and provide lead compounds for further mechanistic study and drug development. In summary, these experiments further validated NSC194598 as potent inhibitor of p53 DNA binding, providing a first-in-class chemical probe and benchmark compound for the research community that studies p53 therapeutic targeting. The assays established for the study of NSC194598 will facilitate the analysis of new compounds identified from the high throughput screen.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting.

Appendix H: William G. "Bill" Bankhead, Jr., and David Coley Cancer Biomedical Research Program Fiscal Year 2022-2023 Closed Grants Funded Fiscal Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
9BC03	Florida State University	Jennifer Steiner, PhD	$732,238.00	11/30/22	Yes	No	Yes
9BC04	Florida State University	George Rust, MD, PhD	$800,487.00	9/30/22	No	No	Yes
9BC13	University of Miami	Kerry L. Burnstein, PhD	$801,000.00	10/31/22	No	No	Yes
9BC14	University of South Florida	HongYuan (Rays) Jiang, PhD	$801,000.00	10/31/22	No	No	Yes

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

Principal Investigator: Jennifer Steiner, PhD

Organization: Florida State University

Summary: Colorectal cancer is among the most prevalent cancers and is the second leading cause of cancer related death. Frequent drinking of moderate to high levels of alcohol increases cancer risk. Cancer cachexia is present in ~50% of colon cancer patients and is characterized by the loss of skeletal muscle and fat mass which directly contributes to decreased muscle strength, quality of life, and treatment compliance and efficacy, as well as increased mortality. Lifestyle factors including alcohol intake, as well as treatments like chemotherapy, may worsen the development of cancer cachexia. The purpose of this project is to determine the impact of alcohol intake on cachexia development as well as the molecular changes incurred by either the prior and/or continued intake of alcohol at tumor initiation. An additional aspect of this work is to investigate the functional impact alcohol may have on skeletal muscle performance in animals suffering from cancer cachexia as muscle weakness can greatly decrease quality of life. These research questions are currently being addressed using a mouse model of cancer cachexia in which colon cancer cells are 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 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 are complete 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: Steiner, J, PI. NIP department in the college of Health and Human Sciences at Florida State University. Influence of obesity and/or gut microbiome on alcohol enhanced cancer cachexia.01/01/2023-12/31/2023. Total Funds Awarded: $10,000.00.

Steiner, J, PI. Pfizer Grant Mechanism: ASPIRE-Cancer Cachexia focus 07/01/2023-06/31/2025. Total Funds Requested: $249,996.00. Pending.

Collaborations: None at the time of reporting.

Journals: Laudato J.A., Tice A, Johnson B, Russo A, Rossetti M, Gordon BS, Steiner JL. Reductions in skeletal muscle protein synthesis and mTORC1 signaling are exaggerated by alcohol use prior to cancer cachexia. 2022 Under Review.

Laudato J.A., Tice A, Johnson B, Russo A, Rossetti M, Gordon BS, Steiner JL. Reductions in skeletal muscle protein synthesis and mTORC1 signaling are exaggerated by alcohol use prior to cancer cachexia. 2022 Under Review

Laudato JA, Tice AL, Call JA, Gordon BS, Steiner JL. Effects of alcohol on skeletal muscle contractile performance in male and female mice. *PLoS One*. 2021;16(8):e0255946. Published 2021 Aug 12. doi:10.1371/journal.pone.0255946.

Laudato JA, Tice AL, Johnson BR, et al. Impact of prior alcohol use on the subsequent development of cancer cachexia in male and female mice. *Alcohol Clin Exp Res* (Hoboken). 2023;47(7):1271-1282. doi:10.1111/acer.15100.

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

Summary: 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 United States, the racial gap in breast and colorectal cancer deaths has paradoxically widened. Minority and disadvantaged populations face barriers to obtaining cutting-edge screening, diagnosis, and treatment in different ways across diverse Florida communities. The purpose of this project is to help each community to understand where they can most strategically target local interventions to achieve the greatest impact on cancer outcomes. This project faced unprecedented challenges in that most of it occurred during the height of the COVID pandemic. This severely limited the ability to conduct face-to-face meetings with community groups in particular, and the research team had to build collaborative effectiveness in virtual team meetings and asynchronous communications. Research staff adapted to all these challenges in various ways. Working through partners who have high trustworthiness, reputations, and relationships in various African American communities across Florida, the research team conducted community conversations, focus groups and stakeholder interviews mostly in virtual space (Zoom or Teams), with some face-to-face stakeholder interviews occurring at the end of the project. For analytic expertise, the research staff built a strong collaboration between the research team and other university personnel and departments.

Because the number of cancer deaths in minority persons in low-population counties was often too low to create stable survival rates, the research staff also ran analyses at the level of cancer regions defined by the Regional Cancer Control Collaboratives. This also will allow researchers in the future to have an outlet of dissemination to those actively engaged in efforts to eliminate cancer disparities in Florida.

Follow on Funding: None at the time of reporting.

Collaborations: The research team has built an effective inter-disciplinary, collaborative team including faculty at the Florida State University (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. After the loss of a full-time investigator (Dr. Luo) last year, the research staff added another analyst to the team. The research team also built collaboration with Dr. Penny Ralston, PhD Professor, Dean Emeritus (College of Health & Human Sciences) & Director, Center on Better Health and Life for Underserved Populations. Dr. Ralston led the community engagement component of the project, in partnership with Mr. Charles Griggs and connections with community organizations such as the 100 Black Men and the Urban League. The research team has 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. The students are actively contributing to discussion, literature review, and manuscript writing.

Journals: Gerend MA, Bradbury R, Harman JS, Rust G. Characteristics Associated with Low-Value Cancer Screening Among Office-Based Physician Visits by Older Adults in the USA. J Gen Intern Med. 2022;37(10):2475-2481. doi:10.1007/s11606-021-07072-1.

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;9(1):35. Published 2021 Sep 24. doi:10.1007/s13755-021-00165-5.

LeBlanc G, Lee I, Carretta H, Luo Y, Sinha D, Rust G. Rural-Urban Differences in Breast Cancer Stage at Diagnosis. Womens Health Rep (New Rochelle). 2022;3(1):207-214. Published 2022 Feb 14. doi:10.1089/whr.2021.0082.

McRoy L, Epané J, Ramamonjiarivelo Z, Zengul F, Weech-Maldonado R, Rust G. Examining the relationship between self-reported lifetime cancer diagnosis and nativity: findings from the National Health and Nutrition Examination Survey (NHANES) 2011-2018. Cancer Causes Control. 2022;33(2):321-329. doi:10.1007/s10552-021-01514-1.

Lee I, Luo Y, Carretta H, LeBlanc G, Sinha D, Rust G. Latent pathway-based Bayesian models to identify intervenable factors of racial disparities in breast cancer stage at diagnosis [published online ahead of print, 2023 Sep 13]. Cancer Causes Control. 2023;10.1007/s10552-023-01785-w. doi:10.1007/s10552-023-01785-w.

Patents: None at the time of reporting.

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

Principal Investigator: Kerry L. Burnstein, PhD

Organization: University of Miami

Summary: Based on the successful innovations employed to complete the project, a manuscript is in preparation that will provide a resource to researchers performing medium-throughput drug screens: Leveraging real-time imaging and automation to enhance medium-throughput prostate cancer drug screens. Two different computational approaches were used to predict the drugs for prostate cancer (PC): the Connectivity Map (CMap) Approach and The Machine Learning Approach. To evaluate the two prediction models and their accuracy to predict compounds sensitivity at the cell line specific level, the cell line activity data and the CMap and machine learning (ML) predictions were integrated and evaluated. This evaluation will continue to be systematically performed for each of the 49 tested compounds and will determine which of the two approaches developed by research staff–CMap or ML–performed best in predicting sensitivity at the cell line level. This analysis will be assembled into a research paper and a web resource. This public resource will provide PC researchers with a platform to query PC gene signatures and predict compound sensitivity. For the production of patient-derived xenografts (PDXs), established PDX models were provided to Sylvester Comprehensive Cancer Center's Cancer Modeling Shared Resource by Dr. Eva Corey at the Genitourinary Cancer Research Lab at University of Washington. Thus far, researchers have demonstrated growth of patient-derived explants (PDEs) in culture (as indicated by the presence of positive Ki67 cells) and confirmatory markers were detected (human androgen receptor (AR) positive cells detected by immunohistochemistry IHC in the PDE cultures; pan-cytokeratin detected as a marker for human tumor cells). Over the course of this project a large amount of data for prostate cancer has already been curated and organized. These data will be made publicly available for the benefit of the research community and to maximize the impact of this project. To enable others to analyze this diverse resource, a Shiny app will be developed to provide user friendly access to the datasets and the ability to browse and visualize the data. The principal investigator and project staff expect this to become an important resource for the prostate cancer research community.

Follow on Funding: None at the time of reporting.

Collaborations: Kerry Burnstein, PhD, University of Miami (PI): overall conduct and management of the project including experimental design; data interpretation and accurate presentation in progress reports and publications. Stephan Schürer, PhD, University of Miami (collaborator): leads computational discovery; project management; data interpretation and accuracy of data in all presentations, progress reports and publications. Vasileos Stathias, PhD, University of Miami (assistant scientist): gene expression modules and disease signatures; mapping tumor models to patient gene expression signatures; drug synergy analyses. Rimpi Khurana, PhD, University of Miami (post-doc): RNAseq processing and analytics including gene set enrichment and network analysis; computational algorithms identifying gene co-expression networks. Beronica Ocasio, University of Miami (graduate student): multi-parametric prioritization and search algorithms to identify best drugs and drug combinations for testing in prostate cancer cells and PDX models. Maria Julia Martinez, PhD, University of Miami (post-doc): all cell-based studies and tumor analysis. Nahuel Peinetti, PhD, University of Miami (post-

doc): tumor analysis (xenografts; ex vivo analysis of tumors). Benjamin Sherman, University of Miami (lab manager): supply ordering; experimental support.

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:10.1016/j.simpa.2022.100257.

Issa, NT, Stathias, V, Schürer, S, Dakshanamurthy, S. Machine and deep learning approaches for cancer drug repurposing. *Semin Cancer Biol*. 2021 Jan;68:132-142. doi:10.1016/j.semcancer.2019.12.011. Epub 2020 Jan 3.

Essegian, D, Khurana, R, Stathias, V, Schürer, SC. The Clinical Kinase Index: A Method to Prioritize Understudied Kinases as Drug Targets for the Treatment of Cancer. *Cell Rep Med*. 2020 Oct 20;1(7):100128. doi:10.1016/j.xcrm.2020.100128.

Essegian, DJ, Chavez, V, Bustamante, F, Schürer, SC, Merchan, JR. Cellular and molecular effects of PNCK, a non-canonical kinase target in renal cell carcinoma. *iScience*. 2022;25(12):105621. Published 2022 Nov 17. doi:10.1016/j.isci.2022.105621.

Peinetti, N, Bilusic, M, Burnstein, KL. Is androgen receptor activity in metastatic prostate cancer a good biomarker for bipolar androgen therapy?. *J Clin Invest*. 2022;132(23):e165357. Published 2022 Dec 1. doi:10.1172/JCI165357.

Patents: None at the time of reporting.

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

Principal Investigator: HongYuan (Rays) Jiang, PhD

Organization: University of South Florida

Summary: 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 cancer universal (present in all cancers), cancer essential (cancer cells require it), and, cancer specific (absent in normal cells). The interdisciplinary team determined that metastatic cancer cells exhibit heme overdrive, based on gene editing loss-of-function studies, the cancer tumor microenvironment exhibits heme overdrive, based on the researchers’ experimental validation of lung cancer-associated fibroblasts isolated from treatment-naïve patients, end-stage solid tumors have elevated heme overdrive, and 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). Dr. Ferreira (USF) has close collaborations with Dr. Jiang, and this work would not be possible without the weekly joint lab meetings and very frequent discussions. The lab members work together and cross-train each other with different set of expertises. Dr. Ferreira's biochemistry expertise and in-depth knowledge in iron and heme metabolisms are essential for the carrying out the project. Dr. Ferreira's general research area relates to enzymology and protein chemistry and the research on heme and iron metabolism is internationally recognized. The Ferreira lab has a broad and solid set of expertise in protein chemistry, proteomics and protein engineering, which are critical to the implementation of the proposed project. Dr. Reuther's (MOFFIT Cancer Center) interest in cell signaling pathways, particularly in the field of myeloid leukemia. The laboratory at MOFFIT has also studied mechanistic aspects of neoplastic janus kinase (JAK) activation, signaling, and therapeutic intervention. In this project, together with Dr. Reuther, researchers have test the hypothesis that erythroid malignancy is inextricably linked with a high level of dependence of heme biosynthesis in cancer progenitor cells. Dr. Reuther has directed experiments described in order to identify potential therapeutic targets for cancers associated with deregulated heme production, such as acute myeloid leukemia. Dr. Reuther has succussuly generated and validated a set of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) knockout (KO) mutants. Subject to periodic review due to statutory requirements Dr. Sebti (VCU) is a recognized cancer researcher who holds the NCI Outstanding Research award. Dr. Sebti has an excellent working relationship and recently co-published a Nature Communication article on cancer drug discovery with the P.I.. This BHC project benefits from his rich drug discovery experience and advice. Dr. Sebti will contribute to experimental design, results interpretation and manuscript preparation. Dr. Sebti has particpated in the seminar and discussions; and the lab will collborate further on drug testing and animal model experiments. The reseach staff have presented the following talk: 'Understanding host heme metabolism in infectious diseases and cancers', M4 meeting, Johns Hopkins All Children's Hospital research campus, St. Petersburg, FL, March 2022.

Journals: Adapa, SR, Hunter, GA, Amin, NE. Heme overdrive rewires pan-cancer cell metabolism. *bioRxiv*. 2022.02.18.481061. doi:10.1101/2022.02.18.481061.

Stojanovski, BM, Hunter, GA, Na, I, et al. 5-Aminolevulinate synthase catalysis: The catcher in heme biosynthesis. *Mol. Gen. Met.* 2019;128(3):178-189. doi:10.1016/j.ymgme.2019.06.003.

Patents: Researchers have started the process of filing a provisional patent entitled "Targeting cancer iron addiction and heme overdrive with chemomodulators".

Appendix I: James and Esther King Biomedical Research
Fiscal Year 2022-2023 Newly Awarded Active Grants
Funded Fiscal Year 2022-2023

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
23K01	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Damon J. Vidrine, DrPH, MS	$1,427,441.00	3/31/26	No	No	No
23K02	H. Lee Moffitt Cancer Center and Research Institute, Inc.	William Douglas Cress, PhD	$569,400.00	3/31/26	No	No	No
23K03	University of Florida	Daiqing Liao, PhD	$569,400.00	3/31/26	No	No	No
23K04	University of Florida	Scott Thomas Robinson, MD, PhD	$569,400.00	3/31/26	Yes	No	No
23K05	University of Florida	Satya Narayan, PhD	$569,400.00	3/31/26	No	No	No
23K06	University of Florida	Brian K. Law, PhD	$568,499.00	5/31/26	Yes	Yes	No
23K07	University of Florida	Shahabeddin Vahdat, PhD	$565,489.00	3/31/26	No	No	No
23K08	University of Miami	Taghrid Asfar, MD, MSPH	$1,423,500.00	9/30/27	No	No	No
23K09	Mayo Clinic Florida	Alan P. Fields, PhD	$569,400.00	3/31/26	No	No	No

1. **Grant#:** 23K01 Creation of an Infrastructure to Support Delivery of mHealth Interventions for Cancer Patients Throughout Florida

Principal Investigator: Damon J. Vidrine, DrPH, MS

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: Providing tobacco cessation treatment to cancer patients is crucial for improving cancer treatment efficacy, recurrence, progression, and quality of life. However, engaging cancer patients in tobacco treatment is challenging, and cessation interventions to date have demonstrated minimal efficacy. Smartphone ownership has become ubiquitous, thus mobile health (mHealth) interventions have the potential to greatly extend reach, as well as to facilitate widespread adoption and sustainability of cessation treatments. Moreover, with the ability to efficiently deliver new treatments, support novel designs, and collect unique data streams, mHealth approaches have the potential to significantly advance the tobacco research field. The goal of this infrastructure grant is to build an mHealth resource to facilitate the creation of apps to address the unique needs of cancer patients and to support the dissemination of these interventions throughout Florida. Currently, existing mHealth resources available to Florida-based researchers are extremely limited. Thus, the proposed infrastructure would fill a critical gap. A key feature of the proposed infrastructure is the ability to simultaneously support diverse projects led by researchers throughout Florida. Other features include the ability to rapidly develop and deliver customizable participant-facing apps created with UX/UI design expertise; flexible crossplatform/device design; centralized data storage; and real-time data access. With the new infrastructure, the research team will conduct demonstration projects to advance research on smoking cessation assessment and intervention approaches among cancer patients. The first part of this project will involve testing mHealth strategies to effectively assess and recruit cancer patients who smoke, while the second part of the project will demonstrate the research team's ability to connect patients to existing resources (e.g., Tobacco Free Quitline) as well as to a newly developed digital intervention. Finally, to demonstrate the unique scientific contributions made possible by the infrastructure, the research team will leverage the unique expertise in machine learning to analyze data (e.g., self report, sensor, activity, etc.) collected

via this new infrastructure. To aid in the recruitment of cancer patients across Florida and to ensure the development of an infrastructure with wide dissemination potential, H. Lee Moffitt Cancer Center and Research Institute, Inc. (Moffitt) will collaborate with investigators from two other major cancer-focused academic institutions in Florida. The investigative team will be complemented by a Scientific Advisory Board comprising scientists across the State in the areas of mHealth, tobacco, oncology, and/or health disparities. Importantly, each center will leverage their expertise in community engagement to assemble a Community Advisory Board that will ensure that the platform and demonstration projects reflect the needs and preferences of both community members and patients throughout 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.

2. **Grant#:** 23K02 Understanding the mechanisms by which STK11 loss in lung cancer leads to immune invasion

Principal Investigator: William Douglas Cress, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: The serine-threonine kinase 11 a.k.a. liver kinase B1 (STK11) protein is a well-characterized regulator of energy metabolism, but its role in immune suppression is poorly understood. The proposed work will test the hypothesis that STK11 mutations leads to the silencing of critical immune signaling molecules and metabolites resulting in immune evasion and seek to identify therapeutic approaches that will counteract these immune resistance mechanisms directly benefiting thousands of lung cancer patients in Florida. Lung cancer is by far Florida's leading cancer killer according to the Florida Cancer Registry. The American Cancer Society publication Cancer Facts and Figures (ACS, 2020) reports that men and women who smoke are 25 times more likely to develop lung cancer than nonsmokers. Deletion or loss-of-function mutations in STK11 in lung adenocarcinoma are strongly correlated (with smoking (odds ratio of 5.0 smokers/non-smokers, $p < 0.01$). Unfortunately for them, numerous studies have found that patients with STK11 mutations (about one in ten of all lung cancer patients) have low immune responses and are less likely to benefit from mmunotherapies (such as Nivolumab/Opdivo and Pembrolizumab/Keytruda) which can provide long-term benefit for many lung cancer patients. Although this grant just received funding three months ago, this team has made significant progress which is detailed the scientific progress report. Most importantly the team has obtained preliminary data in mouse models that support the hypothesis that an Food and Drug Administration-Approved drug, difluoromethylornithine, that inhibits the enzyme ornithine decarboxylase 1 will synergize with standard of care immunotherapies, such as Nivolumab/Opdivo and Pembrolizumab/Keytruda, in lung cancer patients with STK11 mutations that would not usually benefit. These data in mice, will make it easier for researchers to attract funding that will help move this drug combination into human patients. By the end of the first 12-18 months of this grant, the team expects to be in position to begin clinical trials in patients. This will greatly benefit Floridians with STK11 mutant lung cancer who will have easy access to this novel therapeutic combination.

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#:** 23K03 Oncogenic signaling that promotes lipid synthesis and resistance to ferroptosis

Principal Investigator: Daiqing Liao, PhD

Organization: University of Florida

Summary: Breast cancer (BC) is the most frequently diagnosed cancer and the second leading cause of cancer-related death in women. BC metastasizes to several distant organs including brain, bones, liver, and lungs. Metastatic disease is the main cause of death in BC patients and clinical outcome and long-term survival rate for patients with such disease remains dismal and has not significantly improved over the past several decades. Increased lipid synthesis has emerged as a key feature for BC metastasis in the brain. Triple-negative BC (TNBC) is a major subtype of BC, accounting for ~15% of all BC cases. TNBC is characterized by the lack of hormone receptor (estrogen and progesterone receptor) expression and HER2 amplification. TNBC has high rates of mortality, primarily due to its propensity to metastasize to distant organs early in the clinical course. Lipogenesis is regulated by two related transcription factors known as sterol regulatory element-binding proteins (SREBP1 and SREBP2). Oncogenic drivers such as RAS and mTOR signaling cascades have been shown to regulate lipogenesis. Regulated lipid synthesis is critical to cancer cell proliferation while preventing cell death. A form of regulated cell death known as ferroptosis is intimately linked to aberrant lipid metabolism. In breast cancer and other cancer types, genes in the lipogenesis pathways are highly upregulated but the mechanisms that control their expression remain incompletely understood. The researchers have identified the chromatin regulator DAXX as a potential key regulator of lipid synthesis in breast cancer. The goal of this project is to define the molecular mechanism and signaling pathways that promote DAXX-mediated lipid synthesis and resistance to ferroptosis in triple negative breast cancer models. In this project, the researchers will use current molecular, cellular and bioinformatics approaches and animal models to elucidate the mechanism by which DAXX controls lipid synthesis and prevents ferroptosis under the regulation of RAS and mTORC1 signaling in BC cells. This project will provide new knowledge in cancer biology and potential rationale for new treatments for patients with breast and other cancer types. Importantly, this project will also provide training opportunity for next generation of scientists and physicians including undergraduates, graduate students, and post-doctoral associates.

Follow on Funding: None at the time of reporting.

Collaborations: Nikee Awasthee, PhD, postdoctoral associate (Daiqing Liao, Mentor), University of Florida Wenlin Yang, PhD, postdoctoral associate (Daiqing Liao, Mentor), University of Florida Chengcheng Meng, M.S. graduate student (Daiqing Liao, mentor), University of Florida Seth Hale, PhD graduate student (Daiqing Liao, mentor), University of Florida Aaron Chait, MS graduate student (Daiqing Liao, mentor), University of Florida Brandon Kim, MS graduate student (Daiqing Liao, mentor), University of Florida

Journals: None at the time of reporting.

Patents: None at the time of reporting.

4. **Grant#:** 23K04 The impact of e-cigarette exposure on skeletal muscle function in peripheral arterial disease

Principal Investigator: Scott Thomas Robinson, MD, PhD

Organization: University of Florida

Summary: Peripheral arterial disease (PAD) involves the progressive narrowing or blockage of blood vessels in the legs. It can lead to debilitating leg pain or, in its most severe form, amputation. Exposure to tobacco smoke increases the risk of developing PAD, makes the disease worse, and reduces the effectiveness of treatments. While the risk of cigarette smoke on PAD is well established, the effect of e-cigarette smoking on the development and progression of PAD is unknown. E-cigarettes contain fewer toxins than tobacco smoke, but more and more studies have shown that the same harmful pathways activated by tobacco smoke are also activated by e-cigarettes. Unfortunately, E-cigarette use has grown dramatically among adolescents and young adults, leading to earlier exposure and leaving many questions about the risk of these individuals developing PAD. Understanding how e-cigarette use impacts PAD is critical to Floridians, as 7.5% of Florida adults report recent E-cigarette use, which is double the national average of 3.7%. E-cigarette use in young adults in Florida is equally alarming, with 21.6% of Florida's high school students reporting current e-cigarette use compared to 11.3% of high school students nationally. In this proposal, the researchers will try to understand the biological mechanisms linking e-cigarette use with worsening peripheral arterial disease. The researchers will examine the effect of e-cigarette use on the different cell types in the body including blood vessel cells, muscle cells, and stem cells. Using this information, the researchers hope to identify targets that can halt or reverse the harmful effects of e-cigarette smoke on the development of PAD. The researchers work thus far has focused on perfecting the models used in the laboratory to study this problem. The researchers are developing a system that allows researchers to take individual cells from different parts of the body, grow them in special dishes, and mimic the conditions of e-cigarette smoke in a laboratory setting. This allows the research team to understand exactly how each cell's function changes in response to e-cigarette smoke. The researchers are also developing a mouse model that allows researchers to expose a whole organism to e-cigarette smoke. The researchers can then study these mice to determine how e-cigarettes contribute to PAD. This project is still in the very early stages but has the potential to provide significant benefit to Floridians by helping understand how PAD is impacted by e-cigarette smoking.

Follow on Funding: Harding, A, PI. Student Research Award Proposal. Society for Vascular Surgery 6/1/2023-9/1/2023 Total Funds Requested: $3,000. Pending.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

5. **Grant#:** 23K05 Novel therapeutic development for breast cancer

Principal Investigator: Satya Narayan, PhD

Organization: University of Florida

Summary: Researchers aim to establish the mechanism of biisoquinolinederivative (DH20931)-induced lipotoxic endoplasmic reticulum (ER)-stress, activating transcription factor 4 (ATF4)/ C/EBP homologous protein (CHOP)/P53 up-regulated modulator of apoptosis (PUMA) pathway activation and coordination with doxorubicin (DOXO)-mediated nuclear function and apoptosis in breast cancer cells. The researchers have made significant progress. The project was able to recruit a graduate student and a postdoctoral fellow for these studies. First, the researchers decided to establish a clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9-mediated ceramide synthase 2 (CERS2)-knockout metastatic adenocarcinoma of the breast (MDA-MB468) and 4T1 cell lines. The researchers have now completed the knocking out experiments and successfully obtained 4T1-CERS2-/- cells. The researchers are still working on the single clone selection of the MB468-CERS2-/- cells. The researchers hope to establish these cells soon as well. The CERS2-knockout cells are key for the completion of future experiments. By using 4T1-CERS2+/+ and 4T1-CERS2-/- cells, the researchers have determined the targeting of DH20931. The researchers hypothesis is that DH20931 interacts with and stimulates CerS2 activity. Thus, the response of DH20931 will be reduced in CerS2-knockot TNBC cells. Indeed, the researchers results showed a significant sensitization of 4T1-CERS2 +/+ cells to DH20931 treatment than to 4T1-CERS2-/- cells in MTT-cell survival and clonogenic assays. Further, using these cell lines the researchers performed a synergy experiment of DH20931 and DOXO. The researchers results showed that 4T1-CERS2-/- cells lost the synergy effect of DH20931 and DOXO that was seen with 4T1-CERS2+/+ cells. These results together suggest that CerS2 expression is critical for the DH20931-dependent sensitization of 4T1 cells. The researchers have also tested the in vitro efficacy of few breast cancer drugs, such as carboplatin, gemcitabine, and paclitaxel in combination with DH20931. The researchers results showed a significant loss of cell survival with all these combinations. While the researchers current focus is on DH20931 and DOXO, in future studies the researchers will explore the mechanism of these drugs as well. AIM 2: Determine in vitro and in vivo ADME and pharmacokinetics of DH20931 and DOXO. In collaboration with Dr. Abhisheak Sharma, the researchers have started the ADME experiments. Results are awaited before researchers can determine in vivo efficacy of DH20931 and DOXO treatments in a patient-derived breast tumor xenograft model.

Follow on Funding: None at the time of reporting.

Collaborations: Haritha Nair, PhD, Postdoctoral Fellow (Satya Narayan, Mentor), University of Florida Hissah Alatawi, MS, Graduate Student (Satya Narayan, Mentor), University of Florida Christopher Vulpe, PhD, Collaborator, University of Florida Abhisheak Sharma, PhD, Collaborator, University of Florida.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

6. **Grant#:** 23K06 Reducing racial disparity in breast cancer survival with a novel synthetic-lethal strategy

Principal Investigator: Brian K. Law, PhD

Organization: University of Florida

Summary: The studies are progressing as planned and on schedule. The Specific Aims of this project are to: identify the disulfide bond(s) that maintain Death Receptor 5 (DR5) in an auto-inhibited state and that are disrupted by Disulfide bond Disrupting Agent (DDA) treatment, determine which subset of DDA Protein Disulfide Isomerase (PDI) target proteins control formation of the regulatory DR5 disulfide bonds, and evaluate the efficacy of DDA-based therapy against Triple-Negative Breast Cancers (TNBCs) from ascorbic acid (AA) or erythorbic acid (EA) patients in animal models. Progress has included mutagenesis studies to demonstrate that mutation of cysteine (Cys) residues in the DR5 auto-inhibitory domain causes full DR5 activation and stabilization in the absence of ligand stimulation. Additional efforts included ribonucleic acid (RNA) sequencing analysis of a triple-negative breast cancer (TNBC) line from an AA patient to characterize its transcriptome and verify the mutations identified in the original tumor sample, including breast cancer gene 2 (BRCA2) mutation. An unexpected discovery was that if the Integrated Stress Response (ISR) is overcome by inhibition of the protein kinase RNA-like endoplasmic reticulum kinase (PERK), several additional endoplasmic reticulum (ER) stress inducers, including Thapsigargin and Tunicamycin alter DR5 disulfide bonding and cooperate with PERK inhibition to induce cancer cell death. This result indicates that DR5 disulfide bonding may be a general sensor and effector of protein folding stress. Studies going forward will employ established and new AA and EA TNBC lines to investigate the efficacy of DDAs in animal models and to determine which subset of PDIs regulate DR5 disulfide bonding. Although not originally proposed, new results suggest that ISR inhibitors such as the compound integrated stress response inhibitor (ISRIB) may synergize with DDAs to induce tumor regression and will be pursued further in both in vitro and in vivo studies. Due to its aging population, Florida has a disproportionately high burden of cancer incidence and mortality. The potential benefit of these studies to Floridians is the production of new anticancer agents for the treatment of aggressive subtypes of breast cancer and new insights into how to selectively kill cancer cells without harming normal tissues.

Follow on Funding: Tirosh, B, PI; Mechanisms of adaptation to chronic ER stress by the PDI network. NIH R01 12/01/2023-11/30/2028. Pending.

Collaborations: Ronald K. Castellano, PhD, Professor, University of Florida (UF) Department (Dept) of Chemistry: Dr. Castellano's laboratory synthesizes the DDA anticancer compounds under study in the project and has produced new DDAs since the project began that are currently being evaluated. Coy D. Heldermon, MD, PhD, Associate Professor, UF Dept. of Medicine: Dr. Heldermon collaborates on the animal studies and together with the Law laboratory are evaluating DDA anticancer activity in vivo and are developing new models of Triple-Negative Breast Cancers (TNBCs), with the ultimate goal of using DDAs against TNBCs in African American patients to reduce the breast cancer survival disparity experienced by African American women.Roberto Sitia, PhD, Professor, Managing Director, Università Vita-Salute San Raffaele, Italy: One of the DDA target proteins is the disulfide isomerase endoplasmic reticulum resident protein 44 (ERp44). In fact, DDAs are the only reported inhibitor of Erp44. Dr. Sitia is a world expert on the role of Erp44 in protein folding and has requested some DDA compound for studying the effects of pharmacological inhibition on the cycling of

Erp44 between the endoplasmic reticulum and Golgi compartments within the secretory pathway. Boaz Tirosh, PhD, Professor, Dept. of Biochemistry, Case Western Reserve University, Cleveland Ohio: Dr. Tirosh discovered the process of "selective Endoplasmic Reticulum retention" (sERr) in which proteins become arrested in the ER in high-molecular mass disulfide-bonded complexes orchestrated by Erp44. In collaboration with Dr. Tirosh, the research staff are determining how DDAs alter sERr by inhibiting Erp44. This collaboration has already involved exchanges of research materials and experimental results.

Journals: None at the time of reporting.

Patents: Law, BK, Castellano, R, Ferreira, R, inventors. NOVEL SMALL MOLECULE ANTICANCER AGENTS. US patent 10,813,904. October 27, 2020.

Law, BK, Jahn, S, et al, inventors; University of Florida Research Foundation, assignee. NOVEL SMALL MOLECULE ANTICANCER AGENTS, COMBINATIONS AND USES THEREOF. US National Phase Application No.: 17/912,477, September 16, 2022; International Filing Date: March 16, 2021; Publication Date: April 20, 2023. Pending.

Law, BK, Castellano, R, inventors; University of Florida Research Foundation, assignee. INHIBITION OF THE PDI FAMILY MEMBERS AGR2, PDIA1, AND ERP44 FOR THERAPEUTIC TREATMENT AND USE IN PREDICTIVE DIAGNOSTICS/MONITORING FOR TREATMENT REGIMENS. US National Phase Application Nos.: PCT/US2022/011961 - WGS Ref.; No.: U1195.70190WO00; International Filing Date: January 11, 2022. Pending.

Law, BK, Castellano, R, inventors; University of Florida Research Foundation, assignee. ANTICANCER COMPOUNDS AND USES THEREOF. US National Phase Application No.: US 63/135,979; Filing Date: January 11, 2022. Pending.

Law, BK, Jahn, S, et al, inventors; University of Florida Research Foundation, assignee. NOVEL SMALL MOLECULE ANTICANCER AGENTS, COMBINATIONS AND USES THEREOF. US National Phase Application No.: US 62/990,544; Filing Date: March 17, 2020. Pending.

Law, BK, Jahn, S, et al, inventors; University of Florida Research Foundation, assignee. NOVEL SMALL MOLECULE ANTICANCER AGENTS, COMBINATIONS AND USES THEREOF. US National Phase Application No.: U1195, 70134US01; Filing Date: June 15, 2018. Pending.

7. **Grant#:** 23K07 The function of spinal cord-brain pathways in spasticity after stroke

Principal Investigator: Shahabeddin Vahdat, PhD

Organization: University of Florida

Summary: This study improves the health of Floridians who have experienced a stroke by providing quantitative neuroimaging markers for evaluating what treatments most directly target the neural origins of spasticity after stroke. The proposed work advances the understanding of the neurobiological basis for poststroke spasticity, test a new neural circuit model for upper limb spasticity in hemiplegic stroke patients, and provide potential quantitative neuroimaging biomarkers to be used as outcome measures in clinical trials. Overall, the proposed projects will expand the foundation of biomedical knowledge related to the diagnosis, treatment, and cure of spastic complications after stroke, and will increase the state's per capita funding for research

by attracting additional funding from outside the state. As the first phase to achieve this aim, the research team has developed and optimized the neuroimaging and processing pipeline and organized the research staff team to achieve these goals. Additionally, two clinicians from the Department of Physical Medicine and Rehabilitation at University of Florida joined the clinical team to identify stroke patients and help with interpretation of clinical results. A list of stroke patients who pass the inclusion criteria has been constructed to be contacted for data collection and the Principal Investigator has interviewed people for the position of the clinical coordinator and has offered the position to a suitable candidate. The scientific progress on this project includes collecting pilot magnetic resonance imaging (MRI) data to test the scanning and stimulation parameters. The scanning and stimulation protocols are optimized and ready to be used in stroke patients. The research team has also completed the processing pipeline for analysis of the simultaneous functional magnetic resonance imaging (fMRI) data of the brain and spinal cord. The PI presented the results in the Organization for Human Brain Mapping International Conference in Montreal, Canada in July 2023.

Follow on Funding: None at the time of reporting.

Collaborations: Two PhD students from the Department of Applied Physiology and Kinesiology at University of Florida are involved and being trained on this research project. Graduate supervision and advisory activities are an important part of the research for training the next generation of neuroscientists who can push the field of movement neuroscience forward. The lab focuses on understanding the neuroplastic mechanisms underlying stroke recovery. To this aim, the lab studies stroke in both rodent models (to gain a mechanistic understanding), and human patients (for clinical translation of findings). As such, two of groups of students, on the pre-clinical and clinical sides, work closely together in this lab to bridge the animal and human stroke research. The team holds regular biweekly lab meetings in which students present new findings in the field and discuss their project. These meetings and continuous interactions between students broaden their perspective on potential clinical applications in different movement disorders. Six undergraduate research volunteers are receiving training and involved in this research project in this lab. The lab provides continuous research training and opportunities for undergraduate students at University of Florida, who are interested in gaining research experience in the field of movement neuroscience. Undergraduate students will join the lab for a period of at least six months and help the graduate students in the lab on different projects.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

8. **Grant#:** 23K08 Expanding The Role Of The Safety Manager To Implement A Workplace Smoking Cessation Program In The Construction Sector

Principal Investigator: Taghrid Asfar, MD, MSPH

Organization: University of Miami

Summary: Since the study start date, the research team held a study kickoff meeting with to discuss study aims as well as co-investigator meetings held to discuss and fine-tune the implementation plan for the study and the management of the cost-effectiveness data collection and analysis. Additionally, the researchers established a Stakeholder Advisory Committee, who

will meet quarterly with the research team to guide and advise on promoting the program and improving its implementation and sustainability. Researchers have developed the study protocol and all study-related materials; screening forms, consent forms, questionnaires such as baseline assessments, and thee-,six-,nine-, and 12-month assessments, the implementation key informant interviews and surveys, and the SMART tobacco treatment manual. The study materials were submitted to the institutional review board (IRB) at the University of Miami (UM) and approved. A study database was created via Qualtrics for data collection. The development of the safety manager training material in the study protocol, human subjects'' protection, smoking cessation treatment, Qualtrics, and participant tracking tools, as well as the Spanish versions of all study materials, including the treatment manual, educational materials, and study flyers were prepared. The ClinicalTrials.gov application for this study is underway. The two research assistant positions have been filled. During this period, several meetings were conducted with leaders and safety managers of eight construction companies who agreed to participate in the study (DPR Construction, Robins & Morton, Skanska, Whiting-Turner, Grycon, Camcon, Kibler, and Lemark). Responses from three other companies (Brodson, ANF, and Coastal) are still pending. During the first meetings, introduction of study objectives was discussed and the creation of an advisory committee for their company. This advisory committee will consist of a study champion, a representative for the safety managers, and a representative for the construction workers. As part of the pre-implementation assessment, the research team recorded and will transcribe and analyze all the meetings with construction companies. Following the first meeting, the researchers developed a checklist for each company to fill out and return. This checklist collects information on the names and contacts information of all members of their advisory committee, the names and contact information of all safety managers who are interested in participating in the study, the locations of all active construction projects, and a list of questions regarding the study logistics such as nicotine patch storage and fax machine access.

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#:** 23K09 Protein Kinase C iota mediated Immune Suppression in Lung Squamous Cell Carcinoma

Principal Investigator: Alan P. Fields, PhD

Organization: Mayo Clinic Florida

Summary: Non-small cell lung cancer (NSCLC) accounts for the majority (80%) of lung cancer diagnoses. NSCLC is divided into two major sub-types, lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). New therapeutic agents targeting major oncogenic drivers of LUAD have led to improved response rates and patient survival. However, due to a lack of well-characterized, validated, and therapeutically actionable oncogenic drivers, similar therapeutic advances for LUSC have not been forthcoming. The introduction of immune checkpoint inhibitors targeting the PD-1/PD-L1 signaling axis (α-PD-1) have resulted in dramatically improved outcomes in a small subset of LUSC patients, especially those exhibiting elevated PD-

L1 expression. However even in LUSC patients selected based on high PD-L1 expression, most do not exhibit a durable response to α-PD-1. Thus, there is an urgent need for better predictive biomarkers of response to α-PD-1, and new therapeutic strategies to improve the durability of response to α-PD1. Research staff have identified PKCι (PRKCI) as an oncogene that drives LUSC tumorigenesis, characterized critical PKCι-dependent oncogenic signaling mechanisms that drive LUSC tumor growth, and identified and characterized a PKCι inhibitor, Auranofin (ANF), that shows clinical promise for treatment of LUSC. More recently, researchers discovered that PKCι creates and maintains a highly immune suppressive tumor microenvironment (TME) that confers resistance to α-PD-1 in a genetically engineered mouse model (GEMM) of LUAD. Interestingly, interrogation of TCGA LUSC dataset reveals that tumors harboring PRKCI copy number gain (CNG) and PRKCI overexpression exhibit genomic signatures consistent with activated PKCι-dependent oncogenic signaling and a highly immune suppressive TME, suggesting that PKCι also confers immune suppression in LUSC. The researchers recently developed the first GEMM of PKCι-driven LUSC with which to directly assess the role of PKCι in LUSC immunity. Based on the published and preliminary data, research staff hypothesize that: 1) PKCι promotes an immune suppressive TME that confers resistance to α-PD-1 in LUSC; 2) drugs targeting PKCι signaling, either alone or in strategic combination, will sensitize LUSC tumors to α-PD-1; and 3) PRKCI CNG, elevated PKCι expression and/or PKCι-dependent signaling intermediates will be useful predictive biomarkers of response of human LUSC tumors to α-PD-1. These hypotheses will be tested through completion of three interrelated specific aims to characterize the immune TME, identify PKCι-dependent immune suppressive signaling mechanisms, and assess α-PD-1 response in the newly developed GEMM of PKCι-driven LUSC, evaluate the ability of drugs targeting PKCι immune suppressive signaling to sensitize PKCι-dependent LUSC tumors to α-PD-1; and assess whether PKCι-related biomarkers predict response to α-PD1 in human LUSC tumors. Successful completion of these aims will enhance understanding of PKCι-mediated immune suppression and α-PD-1 resistance in LUSC, develop novel therapeutic approaches to improve response of LUSC to α-PD-1, and identify better predictive biomarkers of α-PD-1 response in human LUSC patients. Clinical translation of key findings will be facilitated by active clinical development of the PKCι inhibitor ANF.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix J: James and Esther King Biomedical Research
Fiscal Year 2022-2023 Active Grants
Funded Fiscal Year 2021-2022

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
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, Inc.	Jennifer Vidrine, PhD	$1,451,330.00	9/30/26	No	No	Yes
22K03	University of Florida	Bently Doonan, MD, MS	$1,458,000.00	9/30/26	No	No	No
22K05	University of Florida	Daiqing Liao, PhD	$583,200.00	3/31/25	No	Yes	Yes
22K06	University of Miami	Nagaraj Nagathihalli, PhD	$583,200.00	3/31/25	No	No	Yes
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	No	No	Yes

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

Principal Investigator: Owen Ross, PhD

Organization: Mayo Clinic Jacksonville

Summary: Building the Florida Cerebrovascular Disease Biorepository and Genomics Center is an ambitious project to establish a state-wide resource for cerebrovascular physicians, investigators, and patients. The research team enrolled the first participant on September 2, 2022. To date, July 31st, 2023, the study has enrolled 28 participants. The patient cohort include a range of clinical phenotypes including Moya Moya (n=12) and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) patients (n=16). Of those 16 females and 12 males enrolled, two participants are Hispanic ethnicity, two Asian, one Black/African American and 16 Caucasian. All participants were enrolled in Mayo Clinic Florida except for two from the University of Miami. University of Miami was activated to new enrollment on 3/13/2023. The study team has completed all necessary training and received all supplies to start collecting blood samples. University of Florida (UF) Health Jacksonville is working on start-up regulatory documents for Institutional Review board (IRB) approval. The team is currently in discussions with two new sites: University of South Florida, and University of Florida Gainesville. The research team hope to set up collaborations over the coming year to broaden the state-wide nature of the collection and to increase the numbers. The clinical team has recently established the first patient support group for small vessel cerebrovascular disease (CADASIL / Moyamoya Support Group) at the clinic and across North Florida. The team has developed flyers and educational documents for the attendees, and both principal investigators will present to the patients to describe the study efforts and ultimate goals for clinical therapeutic interventions strategies. Finally, the team has begun optimizing lab protocols for genomic DNA analysis including new technologies (long-read whole-genome sequencing) to fully characterize the genes of the enrolled participants. Defining the genetic basis of disease is a critical undertaking of the project, and providing a genomic center across the state for cerebrovascular disease which will allow more broad genomic approaches will help drive the research and repository. Overall, these efforts to build a cerebrovascular resource will directly engage

patients and physicians across the state and provide education on rare cerebrovascular phenotypes and how to handle corresponding genetic information that may support diagnostic and prognostic advice. A successful resource, such as the Florida Cerebrovascular Disease Biorepository and Genomics Center will help lay a solid foundation for Florida medical care and research to build on.

Follow on Funding: None at the time of reporting.

Collaborations: University of Miami

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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, Inc.

Summary: The research team completed significant work towards meeting project goals. First, the research team completed development of all study tools and materials (for example, adapted Motivation And Problem Solving (MAPS) manual to incorporate the digital treatment adjuvant, finalized Research Electronic Data Capture (REDCap) database, developed digital treatment adjuvant text messaging content and delivery schedule). Study staff were hired and trained on all study procedures including delivery of the MAPS counseling intervention. The research team collaborated with Moffitt's Strategic Marketing team to develop an online participant recruitment campaign with Moffitt's advertising partners, Birdsall, Voss and Associates (BVK) and Socius. Recruitment to the pilot study launched on December 8th, 2022. To date, over 700 individuals completed the online pre-screening instrument, and approximately 130 of these appeared to be eligible. Research staff attempted to contact all 130 individuals, successfully completing screenings for 65 individuals; six were successfully enrolled to the pilot study, and 36 were enrolled and randomized to the main trial. The research team is currently expanding recruitment channels to include local print media, recruiting participants via Moffitt clinics and electronic health record, and engaging a professional recruitment company called BuildClinical. Research staff are actively delivering the MAPS intervention and conducting follow up assessments with enrolled participants. To date, of the 36 participants enrolled to the main trial, 27 participants have reached the three-month assessment point and 23 have completed their three-month assessment, which reflects an 85% completion rate; six participants have reached their six-month assessment point and all six have completed this assessment. This reflects a 100% completion rate.

Follow on Funding: None at the time of reporting.

Collaborations: A clinical psychology doctoral student enrolled at the University of South Florida delivered the MAPS intervention to participants. This individual also participated in MAPS training and clinical supervision.

Journals: Vidrine, JI, Fennell, BS, Simmons, VN, et al. Enhancing long-term smoking abstinence among individuals with a history of cervical intraepithelial neoplasia or cervical

cancer (Project ACCESS): protocol for a randomized clinical trial. *BMC Public Health* 23, 1284 (2023). Doi:10.1186/s12889-023-16189-3.

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

Summary: During this past year the researchers made progress in both the design of the clinical trial, its safe conduction, and implemented the necessary oversight and checks and balances to assure strong results. The researchers obtained Institutional Review Board (IRB) approval for the study and added a key feature of an external data and safety monitoring board to serve as both safety and oversight committee but also to evaluate all external documents and communications to mitigate any possible perceived conflict of interest. The researchers added key members to the research team including Dr. Thomas George, a clinical oncologist with 20 years of experience in first in human clinical trials to serve as the conflict of interest monitor for the study and appointed Dr. Eugenio Manso, the head of GMP (Good Manufacturing Practice) facility at the University of Florida to provide expert guidance and oversight in the generation of the nanoparticle. The researchers have conducted GMP test runs on melanoma patient tissue samples to ensure the process is optimized and expedited for generation of patient specific messenger ribonucleic acid (mRNA) vaccines once the researchers recruit the first patient to the study. The researchers have also been performing a parallel first in human phase 0 study in patients with malignant glioblastoma to test the total tumor derived nanoparticle. The researchers have been successful in treating five patients on this study and found valuable insight into the management of the therapy including how to better watch for immune mediated side effects, and how to immediately intervene with therapies to alleviate these symptoms. The study is currently IRB approved and on internal hold as the researchers institute a plan for the first administration of the vaccine in the in patient bone marrow transplant unit once the researchers recruit the first subject. The clinical trial is poised to open in the coming month with patients being identified in clinic as potential study subjects.

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#:** 22K05 Development of first-in-class HDAC3-selective degraders for breast cancer therapy

Principal Investigator: Daiqing Liao, PhD

Organization: University of Florida

Summary: There are several major breast cancer (BC) subtypes: estrogen receptor-(ER)-positive (ER+), 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. The researchers have 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 researchers propose to optimize and validate the HDAC3 PROTACs for potency and selectivity in degrading HDAC3. The researchers will also determine the mechanism of action of these novel HDAC3 PROTACs, their in vivo drug properties, their safety profiles, and their anticancer and anti-metastatic efficacy in pre-clinical 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 accepted for publication in the peer-reviewed journal Cell Chemical Biology, a leading journal in the field of chemical biology. The research team members also presented their findings from the project in the 2023 annual meeting of the American Association for Cancer Research (AACR), the world's premier conference for cancer research, in Orlando, Florida, in April of 2023. 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; MS graduate student (Daiqing Liao, mentor), University of Florida; Seth Hale, PhD graduate student (Daiqing Liao, mentor), University of Florida; Yufeng Xiao, PhD, postdoctoral associate (Guangrong Zheng, Mentor), University of Florida; Yi Liu, PhD, postdoctoral associate (Guangrong Zheng, Mentor), University of Florida.

Journals: Xiao Y, Hale S, Awasthee N, et al. HDAC3 and HDAC8 PROTAC dual degrader reveals roles of histone acetylation in gene regulation [published online ahead of print, 2023 Aug 8]. *Cell Chem Biol*. 2023;S2451-9456(23)00240-4. Doi:10.1016/j.chembiol.2023.07.010.

Patents: Liao, D, Xiao, Y, Zhang, X, Zheng, G, inventors. University of Florida, assignee. Benzoylhydrazide-Derived HDAC Degraders as Therapeutics for Treating Cancer and Other Human Diseases. United states National Phase Patent Application, Application #: 17/925,776, UF Ref. No. UF Ref. No. T18184US002, filed on November 16, 2022.

Liao, D, Xiao, Y, Zhang, X, Zheng, G, inventors. University of Florida, assignee. Benzoylhydrazide-Derived HDAC Degraders as Therapeutics for Treating Cancer and Other

Human Diseases. European Patent Application No. 21807766, UF Ref. No. T18184EP001, filed on December 16, 2022.

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

Principal Investigator: Nagaraj Nagathihalli, PhD

Organization: University of Miami

Summary: Flow cytometric analysis was performed on KPC pancreatic ductal adenocarcinoma (PDAC) tumors to evaluate the expression of the leukemia inhibitory factor receptor (LIFR), the receptor that binds to the LIF ligand. This analysis aimed to determine the presence and abundance of LIFR in various cell types within the PDAC tumor microenvironment (TME). Researchers began flow cytometry-based immunophenotyping analysis conducted on single-cell suspensions derived from KPC PDAC tumors that were generated through syngeneic orthotopic implantation of CREBEV and CREBKO tumor cells in immunocompetent C57BL/6 mice. Hyperactivation of tumor cell autonomous signaling has the potential to modulate the tumor immune microenvironment of pancreatic ductal adenocarcinoma (PDAC) by altering the secretome profile of immunomodulatory cytokines and provide the pathological context to promote therapeutic resistance by recruiting the immunosuppressive innate immune subsets including granulocytes, thereby promoting T cell exclusion. The project's current work has successfully elucidated the tumor cell-intrinsic function of CREB in governing the secretion of a pivotal immunosuppressive cytokine, Leukemia inhibitory factor (LIF). The findings establish that CREB regulates LIF as a vital mediator that orchestrates the recruitment and functional modulation of innate immune myeloid subsets within the tumor microenvironment (TME) of PDAC. A re-analysis revealed a substantial proportion of myeloid cells, including myeloid derived suppressor cells (MDSCs) and macrophages, expressing LIFR compared to other cell types within the TME. This finding establishes myeloid subsets as the primary target population for the immunosuppressive cytokine LIF. Overall, these data reveal that overexpression of cancer cell autonomous CREB has the potential to regulate the trafficking dynamics of neutrophil/myeloid cells in PDAC via LIF-LIFR. In the earlier study, research staff demonstrated that inhibition or deletion of CREB leads to a substantial reduction in tumor burden and showed preliminary analysis with CREB inhibition. Building upon these findings, researchers aimed to investigate and validate previous reports, whether the modulation of innate immunosuppressive myeloid subsets through CREB inhibition has an impact on adaptive immune subsets, particularly T cells. Intriguingly, upon investigation of the T-cell populations by flow cytometric analysis, researchers found a significant increase in the proportion of CD8+PD- 1+ T cells and activated CD8+CD69+ T cell subsets. CREBi also resulted in heightened infiltration of activated CD8+ T cells with effector memory like phenotype (CD44High CD62Llow) as compared to vehicle treated PDAC tumors. Notably, effector memory T cells have been previously established to possess antitumor immunity. Therefore, the profound antitumor benefits observed with CREB loss or inhibition could be associated with the expansion of these immune subsets within the TME of PDAC.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Mehra S, Garrido VT, Dosch AR, et al. Remodeling of Stromal Immune Microenvironment by Urolithin A Improves Survival with Immune Checkpoint Blockade in

Pancreatic Cancer. *Cancer Res Commun*. 2023;3(7):1224-1236. Published 2023 Jul 12. Doi:10.1158/2767-9764.CRC-22-0329.

Patents: None at the time of reporting.

6. **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

Summary: The number of passive and active smokers living with end-stage renal disease (ESRD) who will eventually need a functional vascular access to receive hemodialysis and extend their lives is increasing. The arteriovenous fistula (AVF) created by surgically connecting an arm vein to a nearby artery is the preferred vascular access for hemodialysis. However, AVF failure due to venous stenosis (narrowing) remains a frequent complication for dialysis patients and significantly contributes to hemodialysis costs. Importantly, researchers do not fully understand the biological processes that lead to failure of hemodialysis accesses even after decades of vascular research. The purpose of this project is to investigate the molecular changes caused by smoking in individual cells in veins, and how these changes increase the susceptibility to failure after AVF creation. The fundamental hypothesis is that smoking changes the cellular ecosystem in pre-access veins that is responsible for vascular wall repair and remodeling and that a disequilibrium in those cells prior to vascular surgery increases the risk for stenosis in the AVF. During the first year, research project staff optimized tissue processing to obtain cell suspensions from veins, and successfully completed the single-cell RNA sequencing of 36 human veins and AVFs from chronic kidney disease (CKD) and non-CKD donors. Venous samples from non-CKD individuals were obtained through a research agreement with the Life Alliance Organ Recovery Agency, while CKD veins and AVF samples were obtained from patients undergoing surgery for AVF creation at the University of Miami. The researchers are close to reaching recruitment goals for this aim. The cellular atlas of peripheral veins from non-CKD individuals has been finalized at the single cell resolution (>20,000 cells), including two veins from smokers and two from never-smokers. In the coming months researchers will compare this cellular atlas to that of CKD veins and AVFs to define the contribution of smoking to poor venous remodeling and the interaction of smoking and CKD to changes in cellular ecology. Researchers will also validate these findings in independent venous samples from the vascular tissue biorepository of >800 biopsies at the University of Miami, including current smokers, former smokers, and never-smokers. Importantly, findings from this project are not only applicable to hemodialysis accesses, but also create a foundation for the study of chronic venous diseases, atherosclerotic disease, and other vascular pathologies.

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

Principal Investigator: Ji Li, PhD

Organization: University of South Florida

Summary: Cigarette smoking is a major preventable cause of morbidity and mortality worldwide. It is estimated that over five 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: None at the time of reporting.

Collaborations: May to August 2022, Zehui Li (Junior), Undergraduate Student, Department of Biomedical Engineering, College of Engineering, University of South Florida May to August 2022, Parth Kulkarni (Junior), Undergraduate Student, Department of Biomedical Engineering, College of Engineering, University of South Florida. May 2021-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. January 2022-present, Hao Wang, Postdoc Fellow, Department of Surgery, Morsani College of Medicine, University of South Florida.

Journals: Zoungrana LI, Krause-Hauch M, Wang H, et al. The Interaction of mTOR and Nrf2 in Neurogenesis and Its Implication in Neurodegenerative Diseases. Cells. 2022;11(13):2048. Published 2022 Jun 28. Doi:10.3390/cells11132048.

Patents: None at the time of reporting.

Appendix K: James and Esther King Biomedical Research
Fiscal Year 2022-2023 Active Grants
Funded Fiscal Year 2020-2021

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
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	Yes	No
21K04	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Christine Chung, MD	$1,339,540.00	4/30/26	No	No	Yes
21K05	University of Miami	Carlos Moraes, PhD	$535,840.00	4/30/24	No	No	Yes
21K06	University of Miami	Helen M. Bramlett, PhD	$535,840.00	5/31/24	Yes	No	Yes
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
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	Yes	No	Yes
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

Summary: 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 that 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 decreased expression of normal endothelial vascular regulatory genes and increased expression of inflammation, extracellular matrix remodeling, and stress response genes. There was also a down-regulation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase family members one and four which was associated with increased endothelial reactive oxygen species (ROS) production. CS induced ROS production plays a critical role in enhancing the risk of CA formation and rupture. The mammalian target of rapamycin (mTOR) plays an important but undefined role in CA stability leading to CA rupture. To determine if CS activates endothelial mTOR leading to enhanced CA risk, the lab generated conditional knockout mice which allows for the selective activation or inhibition of mTOR only in vascular endothelial cells. The generation of these breeding colonies took longer than expected, but preliminary data demonstrates that inhibition of mTOR reduces the rate of CS induced CA formation and rupture. 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 coil-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 nitrogen oxides (NOX) expression and how this relates to endothelial dysfunction and cerebral aneurysm pathology based upon the patient's smoking status. During this yearly reporting period the research group has collected six CA tissues, 14 CA blood samples, and 21 endothelial coil-biopsies, and their corresponding control samples. Stratification of the blood samples collected to date reveals that 15% of the patients enrolled are current smokers, while 25% are former smokers, and 60% have never smoked. Enough samples have been collected such that the lab has started isolating ribonucleic acid (RNA) from the blood and tissue samples and will begin gene expression studies using RNA sequencing. 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 who 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

Summary: 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 research team 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. 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: Liao, D, PI. National Cancer Institute, National Institutes of Health, 4/1/2022-3/31/2027, $3,396,730.00.

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

Journals: None at the time of reporting.

Patents: Liao, D, Inventor, University of Florida, assignee. COMPOUNDS AND METHODS OF USE FOR DEGRADING REST COMPRESSOR 1, LYSINE-SPECIFIC HISTONE DEMETHYLASE, HISTONE DEACETYLASE 1 AND HISTONE DEACETYLASE 2 IN THE CoREST COMPLEX. Serial No.:PCT/US22/73187, filed June 27, 2022. Ref No.:T18443WO001 (222111-2290).

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, Inc.

Summary: The most significant scientific accomplishment made from July 1, 2022, to June 30, 2023, is that the research team completed enrolling the patients to the clinical trial and completed tumor collection inventory. In summary, anti-programmed cell death protein 1 (PD-1) therapy is a standard of care in recurrent metastatic head and neck squamous cell carcinoma (RM HNSCC). Vascular endothelial growth factor inhibitors, including tyrosine kinase inhibitors, have immuno-modulatory properties and have offered promising results when combined with anti-PD-1 agents. The research team conducted a phase 2, multicenter, single-arm trial of pembrolizumab and cabozantinib in patients with RM HNSCC who had Response Evaluation Criteria in Solid Tumors v.1.1 measurable disease and no contraindications to either agent. The research team assessed the primary end points of tolerability and overall response rate to the combination with secondary end points of progression-free survival and overall survival and performed correlative studies with PD-L1 and combined positive score, CD8+ T cell infiltration and tumor mutational burden. A total of 50 patients were screened and 36 were enrolled with 33 evaluable for response. The primary end point was met, with 17 out of 33 patients having a partial response (52%) and 13 (39%) stable disease with an overall clinical benefit rate of 91%. Median and one-year overall survival were 22.3 months [95% confidence interval (CI)= 11.7-32.9] and 68.4% (95% CI= 45.1%-83.5%), respectively. Median and one-year progression-free survival were 14.6 months (95% CI= 8.2-19.6) and 54% (95% CI= 31.5%-72%), respectively. Grade 3 or higher treatment-related adverse events included increased aspartate aminotransferase (n = 2, 5.6%). In 16 patients (44.4%), the dose of cabozantinib was reduced

to 20mg daily. The overall response rate correlated positively with baseline CD8+ T cell infiltration. There was no observed correlation between tumor mutational burden and clinical outcome. Pembrolizumab and cabozantinib were well tolerated and showed promising clinical activity in patients with RM HNSCC. The research team is conducting additional investigations to validate these findings. The novel therapy will improve the lives of patients in Florida with currently incurable RM HNSCC.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Lin CN, Chung CH, Tan AC. NuKit: A deep learning platform for fast nucleus segmentation of histopathological images. *J Bioinform Comput Biol*. 2023;21(1):2350002. doi:10.1142/S0219720023500026.

Cao B, Patel KB, Li T, Yao S, Chung CH, Wang X. A subnetwork-based framework for prioritizing and evaluating prognostic gene modules from cancer transcriptome data. *iScience*. 2022;26(2):105915. Published 2022 Dec 30. doi:10.1016/j.isci.2022.105915.

Saba NF, Steuer CE, Ekpenyong A, et al. Pembrolizumab and cabozantinib in recurrent metastatic head and neck squamous cell carcinoma: a phase 2 trial. *Nat Med*. 2023;29(4):880-887. doi:10.1038/s41591-023-02275-x.

Chaudhary R, Slebos RJC, Noel LC, et al. EGFR Inhibition by Cetuximab Modulates Hypoxia and IFN Response Genes in Head and Neck Squamous Cell Carcinoma. *Cancer Res Commun*. 2023;3(5):896-907. Published 2023 May 22. doi:10.1158/2767-9764.CRC-22-0443.

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

Summary: Damage to mitochondrial deoxyribonucleic acid (mtDNA) has been associated to diseases and aging. The main goal of this project is to understand how mtDNA deletions are formed. To achieve this goal, the researchers are studying mtDNA fragmentation and deletion formation, as these two events are linked. The research team has previously shown that mtDNA fragmentation pre-disposes mtDNA molecules to recombine and form deletions. Researchers have analyzed both wild-type controls, DNA polymerase subunit gamma (POLG) exonuclease deficient (mutator) and mitochondrial genome maintenance exonuclease 1 (MGME1) knockout (KO) mice. These enzymes are important for mtDNA replication. The research team found that when these activities are absent, there is an increase in the levels of mtDNA fragmentation. POLG is the replicative polymerase, but its proofreading exonuclease activity also degrades mtDNA fragments. Research staff are also studying how cigarette smoke influences the formation of mtDNA deletions. To the moment, researchers found that smoking increases mtDNA deletions in the brain, but surprisingly, not in lungs.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Walker BR, Moraes CT. Nuclear-Mitochondrial Interactions. *Biomolecules*. 2022;12(3):427. Published 2022 Mar 10. doi:10.3390/biom12030427.

Barrera-Paez JD, Moraes CT. Mitochondrial genome engineering coming-of-age [published correction appears in Trends Genet. 2022 Aug 26]. *Trends Genet*. 2022;38(8):869-880. doi:10.1016/j.tig.2022.04.011.

Shoop, WK, Bacman, SR, Barrera-Paez, JD, et al. Mitochondrial gene editing. *Nat Rev Methods Primers*. 3, 19 (2023).doi:10.1038/s43586-023-00200-7.

Garcia, S, Saldana-Caboverde, A, Anwar, M, et al. Enhanced glycolysis and GSK3 inactivation promote brain metabolic adaptations following neuronal mitochondrial stress. *Human Molecular Genetics*. 3;31(5):692-704 (2022). PMCID: PMC9077416 doi:10.1093/hmg/ddab282.

Pinto M, Diaz F, Nissanka N, et al. Adult-Onset Deficiency of Mitochondrial Complex III in a Mouse Model of Alzheimer's Disease Decreases Amyloid Beta Plaque Formation. *Mol Neurobiol*. 2022;59(10):6552-6566. doi:10.1007/s12035-022-02992-3.

Shoop WK, Gorsuch CL, Bacman SR, Moraes CT. Precise and simultaneous quantification of mitochondrial DNA heteroplasmy and copy number by digital PCR. *J Biol Chem*. 2022;298(11):102574. doi:10.1016/j.jbc.2022.102574.

Lang AL, Nissanka N, Louzada RA, et al. A Defect in Mitochondrial Complex III but Not in Complexes I or IV Causes Early β-Cell Dysfunction and Hyperglycemia in Mice. *Diabetes*. 2023;72(9):1262-1276. doi:10.2337/db22-0728.

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

Summary: 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). 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. Therefore, the question arises; will ECs use impact the outcome of stroke? To answer this question the research team 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 research staff confirmed the nicotinine levels in the brain and achieved levels similar to that of serum in humans. Following establishing the model of EC exposure, 48 mice were randomized to either air or EC exposure for eight days. At the end of EC exposure, either brain tissue was collected from mice for biochemical assay or animals were randomized further to sham or photothrombotic stroke surgery. So far, the staff have exposed 28 animals to the surgery and 13 animals received therapeutic hypothermia. These animals were also tested for motor and cognitive behavior. Importantly these studies are conducted in a double blinded fashion and therefore, quantification of data will be performed once enough samples size is achieved in all the experimental groups. The research staff have also presented the research at several conferences during the past year.

Follow on Funding: Raval, AP, Bramlett, H, Szczesna-Cordary, D, PIs. Institutional Scientific Awards Committee (SAC) interdisciplinary. 11/1/22-11/30/23. Total Funds Requested: $50,000. Pending.

Collaborations: Research staff collaborated with Dr. Danuta Szczesna-Cordary, PhD, Professor of Molecular and Cellular Pharmacology and part of the currently ongoing collaborative grant listed above

Journals: Pradhyumnan H, Reddy V, Bassett ZQ, et al. Post-stroke periodic estrogen receptor-beta agonist improves cognition in aged female rats. *Neurochem Int*. 2023;165:105521. doi:10.1016/j.neuint.2023.105521.
Raval AP, Bramlett HM. Introduction to the special issue on neurological disorders across the female life span. *Neurobiol Dis*. 2022;174:105886. doi:10.1016/j.nbd.2022.105886.

Raval AP, Bramlett HM. COVID-19 Deterred Career Path of Our Undergraduate Neuroscience Students: Educators' Perspective. eNeuro. 2022;9(5):ENEURO.0384-22.2022. Published 2022 Oct 6. doi:10.1523/ENEURO.0384-22.2022.

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

Summary: During the current year, progress was made in all aims. One of the goals in the application was to assess the impact of obesity on chemerin expression in animal models of renal cell carcinoma development, and to optimize the induction of obesity in the C57bl6 model to test for the elevation of Chemerin expression. Research staff completed an assay over a 24-week period and have seen a clear and sustained increase in body weights in the high fat diet animals. The final average weights for the groups were over 40g versus 26 for the normal chow animals. As staff aimed to identify the optimal time to observe elevated chemerin, serum collection from the animals on a bi or tri weekly basis allowed detection of chemerin levels by enzyme-linked immunosorbent assay (ELISA). It was have found a >50% increase in chemerin levels past the 16-week interval, in agreement with the literature. Strikingly, however, the levels

are not maintained over time. There was then a sharp drop off after the 20-week period in the overall levels. Notably, there was still a significant increase in the high fat diet animals compared to the normal chow. The grant also aimed to optimize the mAb approach to inhibiting Chemerin as a therapeutic in clear cell renal cell carcinoma (ccRCC). Staff have performed tumor growth assays on five patient-derived xenograft (PDX) models, with varying expression of Chemerin. Notably, they found three PDX models with high chemerin levels that were dependent on chemerin for sustained tumor growth. In contrast, researchers found PDX models with low chemerin to be insensitive to chemerin inhibitory. Thus, chemerin expression levels appear to be predictive of tumor responses. Research staff next sought to understand the effects on lipid metabolism after chemerin inhibition, and thus performed unbiased lipidomic analyses on the PDX tumor samples. The lipid profiles of untreated control tumors shift significantly after antibody treatments, suggesting that inhibition of chemerin signaling is sufficient to change the lipids in the tumor. This is in accordance with other data demonstrating a reduction in lipid droplet formation in these tumors. Having previously collected primary tumor samples from patients in the clinic, staff were able to assess chemerin levels and dichotomize tumors into low and high chemerin and perform lipidomics on them to ask the question of whether chemerin inhibition in the PDX models could change the lipid profile from a tumor from a high to low chemerin. It was evident that the lipid profile of low and high tumor samples are unique, and that when the PDX lipidome is assessed, the phosphatidylserine, phosphatidylglycerol, phosphatidylethanolamine, and diacylglycerol all revert from high to low levels, mimicking chemerin low 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#:** 21K08 Endothelial Circadian Clock Protein PER1 Modulates Salt-sensitive Hypertension

Principal Investigator: Michelle Gumz, PhD

Organization: University of Florida

Summary: 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. They have recently identified a novel long non-coding RNA, EDN-1 AS, that is regulated by PER1 and increases ET-1 messenger ribonucleic acid (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 Endothelin-1 (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. The research team performed and quantified in situ hybridization (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 and now have sufficient number of mice for the experiments 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

Summary: 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. 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: Transforming growth factor-beta (TGF-beta) is induced by cigarette smoke, TGF-beta suppresses LPO protein in human bronchial epithelial cell line (BEAS2B) airway epithelial cell lines and TGF-beta increases expression of the microRNA (miR)-449b. TGF-beta, which is induced by cigarette smoke suppresses LPO protein in three dimensional (3D) model of bronchial epithelium (bronchial epithelium redifferentiated at the air-liquid interface). TGF-beta increases hydrogen peroxide levels in the team's 3D model of bronchial epithelium. In

preliminary data it was 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. The researchers show that like TGF-beta, miR-449b mimic also impairs mitophagy and promotes senescence in airway epithelial cells. 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 determining the mechanism by which cigarette smoke promotes inflammation. 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. 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

Summary: 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: Xing, C, Salloum, R, PIs. NIH R33, The potential of kava in enabling tobacco cessation - its holistic effects in managing stress and insomnia associated with abstinence 04/01/2023-03/31/2026. Award Notice Date: 07/20/2023. Total Amount Awarded: $522,987.00. CFDA Code: 213.

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

Summary: The purpose of this study is to examine the origins of unresolving inflammation that occurs in obese individuals and to better understand how smoking contributes to increased susceptibility of cardiac damage when blood flow to the heart is completely or transiently halted. Mouse models are being used to understand how obesity and exposure to cigarette smoke increase pro-inflammatory pathways. Project staff are also involved in obtaining blood samples 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. Smoking studies were performed in mice to compare the response of wild type (normal unmodified mice) with sarcospan-deleted male and female mice. A number of conditions are being compared: sex, gene background, obese versus nonobese, smoking versus non-smoking. The next studies by research personnel will examine the effect of smoking on mice eating an unhealthy high fat diet. Removal of the gene sarcospan protects both male and female mice from obesity and insulin resistance. This is favorable, but the sarcospan gene also appears to play a role in regulating inflammation. 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. It has also been shown that autoimmune conditions can be linked to smoking and sarcospan-deleted mice show increased susceptibility to autoimmunity. Autoimmune readouts will be included after mice are smoked to determine whether the combined effect of SSPN deficiency and smoking increases susceptibility to autoimmunity. These studies many uncover unknown genetic risk factors that predispose smokers to autoimmune diseases. This study will provide information on whether compounds that increase sarcospan expression can protect smokers (obese and normal weight) from cardiac damage due to oxidative stress and abnormal immune cell activation. The studies conducted by research staff in mice are being compared to human studies examining obese individuals and/or smokers. In mice - markers will be assessed that report oxidative stress and inflammation in the heart's of sarcospan-deficient mice and wild-type mice after heart attack. In humans, researchers will correlate these markers in blood with levels of sarcospan expression to assess how therapeutic strategies that alter sarcospan levels can reduce cardiac risk of overweight smokers.

Follow on Funding: Parvatiyar, M,Gordon, B, PIs. Florida State University - Institute of Successful Longevity. 05/14/23-05/15/24. Total Funds Awarded: $25,000.00.

Collaborations: Kislay Parvatiyar, PhD - Tulane University (New Orleans, LA) advice on innate immune signaling pathways. Shyam Bansal, PhD, State University (Columbus, OH) assisting with characterizing the immune response of sarcospan-deficient mice to myocardial infarction, training in survival surgeries and echocardiography. Kyle Smith, PhD - Florida State University (Tallahassee, FL) flow cytometry training of sarcospan-deficient mice after smoking. Brad Gordon, PhD. - Florida State University (Tallahassee, FL) muscle mechanics and muscle atrophy.

Journals: Olateju BS, Kahmini AR, Valera IC, et al, Assessment of the Response of Young and Aged Sarcospan-Deficient Mice to Nutrient Excess. *American Physiology Society*. 23 May 2023. doi:10.1152/physiol.2023.38.S1.5735212.

Kahmini AR, Valera IC, Carbonell AC, et al., Compensatory calcium handling may underlie increased arrhythmia susceptibility of sarcospan-deficient mice after ischemia-reperfusion injury, *Biophysical Journal*, vol. 122, issue 3, p. 381a (2023). doi:10.1016/j.bpj.2022.11.2091.

Mumbi F, Vieira ML, Olateju BS, Coscarella IL, Chase PB, Pinto JR and Parvatiyar MS (2023) Investigating the Sarcolemma-Sarcomere Connection in Dictating Force Transmission in the Heart, Biophysical Journal, vol. 122, issue 3, p. 404a (2023). doi: 10.1016/j.bpj.2022.11.2200.

Kwiat VR, Reis G, Valera IC, Parvatiyar K and Parvatiyar MS (2022) Autoimmunity as a sequela to obesity and systemic inflammation. Review. *Front. Physiol*. 21 Nov 2022:13:88702. doi:10.3389/fphys.2022.887702.

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

Summary: The researchers examine, in electronic cigarette (EC) users, the extent to which vascular toxicity is related to endothelial function in the airway, pulmonary and systemic circulations. Each participant has three visits: Screening, measurement of airway blood flow, and echocardiography/flow-mediated vasodilation in the forearm. Initially there was a delay in subject recruitment due to an irreversible breakdown of the mass spectrometer. It had to be replaced and tested. Because of this delay, the research team has changed the protocol that now allows visit two to be done earlier. The research team now includes EC users of any brand containing 5 % nicotine rather than specific brands. The researchers have now changed the protocol such that current vapers that are ex-smokers of <10 pack-years and quit regular smoking over two years ago, and users of other tobacco products less than once a week are now eligible for participation in the study. All protocol changes have been approved by the University of Miami institutional review board (IRB). As of July 24, 2023, the enrollment status is as follows: Screened: 69, Enrolled: six (five controls, one vaper); Completed six (five controls, one vaper).The research team believes that the slow enrollment rate has been caused by the initial brand restriction, the technical issues with the mass spectrometer, and the strict criteria for

not using concurrent tobacco products. Researchers are confident that with the protocol amendments, the research team will be able to achieve full enrollment by the end of the grant. Researchers also investigated the effects of graded EC vapor exposure on cytokine secretion and oxidative stress markers (ROS) in endothelial cell cultures, exposed to increasing concentrations of EC condensates from four different flavors/brands (Juul Menthol, Juul Virginia Tobacco, HQD Ice Mint, and HQD Black Ice) and pure nicotine. So far researchers have found that HQD condensates are more cytotoxic than their Juul counterparts, and there are differences in ROS induction among brands and flavors. EC chemicals induce an imbalance in angiogenic vs. pro-inflammatory cytokines that may affect the ability of cells to respond to various stimuli. Furthermore, EC condensates induce disease-relevant changes in global gene expression. The findings support the noxious effects of vaping on pulmonary and vascular health and provide evidence for a differential contribution of e-cig brands and flavors to endothelial dysfunction. These results were presented at the 2023 American Thoracic Society International Conference. Researchers are currently working on characterizing the differences among the different EC condensates and cigarette smoke condensates by nuclear magnetic resonance (NMR) through a collaboration with ChenomX and Covalent Metrology.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix L: James and Esther King Biomedical Research
Fiscal Year 2022-2023 Active Grants
Funded Fiscal Year 2019-2020

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
20K05	University of Florida	Terence Ryan, PhD	$626,710.00	11/30/23	No	No	Yes
20K06	University of Florida	Gilbert Upchurch, Jr., MD	$626,708.00	11/30/23	No	No	No
20K07	University of Florida	Daiqing Liao, PhD	$626,708.00	11/30/23	No	No	Yes
20K08	University of Florida	Dorian K. Roase, PhD, MS, PT	$688,940.00	5/31/25	No	No	No
20K09	University of Miami	Ami Raval, PhD, MSPH	$626,710.00	11/30/23	No	No	Yes
20K10	University of Miami	Taghrid Asfar, MD, MSPH	$1,253,415.00	4/30/25	Yes	No	Yes

1. **Grant#:** 20K05 Role of the aryl hydrocarbon receptor in tobacco smoke induced skeletal muscle atrophy.

Principal Investigator: Terence Ryan, PhD

Organization: University of Florida

Summary: In this award, the researchers have performed mechanistic discovery based experiments exploring the role of the aryl hydrocarbon receptor (AHR) in the skeletal muscle pathology that develops with tobacco smoking. Using a newly developed AHR mouse line in which the gene was deleted in skeletal muscle, the researchers have discovered that removing the AHR from mice exposed to chronic cigarette smoke resulted in a substantial improvement in mitochondrial function in the skeletal muscle. Interestingly, this improvement was only found in male mice, but not females indicating a sex-dependent effect. Because cigarette smoke can impact more than just muscle and the AHR, the researchers also used a virus to express a mutant AHR that was always active in normal healthy mice. In this experiment, the researchers found that expression of a chronically active AHR in muscle was sufficient to impair muscle mitochondrial function – an observation consistent with male mice exposed to cigarette smoking. Additionally, the researchers found that female mice appeared to have more severe muscle pathology with chronic cigarette smoking compared to males, an unexpected finding.

Follow on Funding: Ryan, T, PI, Linking kynurenine accumulation and AHR activation to exacerbated aging. National Institute of Aging, NIH R01. 05/01/2022-01/31/2027. Award Ntoice Date: 02/10/2023. Total Funds Awarded: $569,891.00. CFDA Code: 866.

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*. 2022;10(3):140. Published 2022 Mar 14. Doi:10.3390/toxics10030140.
Thome T, Miguez K, Willms AJ, et al. Chronic aryl hydrocarbon receptor activity phenocopies smoking-induced skeletal muscle impairment. *J Cachexia Sarcopenia Muscle*. 2022;13(1):589-604. Doi:10.1002/jcsm.12826.

Patents: None at the time of reporting.

2. **Grant#:** 20K06 Role of myeloid-derived suppressor cells in aortic aneurysms and rupture

Principal Investigator: Gilbert Upchurch, Jr., MD

Organization: University of Florida

Summary: Researchers aim 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 IL-17 (interleukin-17) signaling that mediates abdominal aortic aneurysm (AAA) formation. To abrogate these critical cytokine dependent inflammatory pathways, the researchers will analyze if cultured G- and M-MDSCs (with/without nicotine or chronic smoking exposure) can significantly upregulate immunosuppress T cell activation and interleukin-17 (IL-17) secretion. New experiments were conducted where C57BL/6 mice were exposed to cigarette smoking inhalation from 3R4F reference cigarettes (Tobacco and Health Research Institute, University of Kentucky, Lexington, KY) at 150 mg/m3 total smoking particles (TSP). The aortic diameter of mice exposed to smoking after three months was increased compared to age-matched controls. The aortic diameter of mice exposed to smoking after six months was significantly increased compared to age-matched controls. Previously, no significant differences were observed between M-MDSCs (CD11b+Ly6C+Ly6C-) between WT mice treated with nicotine vs. placebo osmotic pumps. Similarly, no significant difference between G-MDSCs (CD11b+Ly6C-Ly6G+) was observed between nicotine vs. placebo osmotic pumps. 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. Similarly, a decrease in G-MDSCs was observed in elastasetreated cebpb null mice compared to elastase-treated Balb/c mice. Previously the researchers have observed that elastase-treatment of WT mice resulted in a multi-fold increase in M-MDSCs (CD11b+Ly6Gly6C+) in aortic tissue of elastase-treated WT mice on day 14 compared to heat-nactivated elastase controls. In a separate experiment, elastase-treated mCXCR2-/- mice (myeloid cell-specific deletion of CXCR2) showed a trend towards decrease in M-MDSCs compared to elastase-treated WT mice. The researchers previous in vitro data shows that treatment of aortic smooth muscle cells (AoSMCs) with transient elastase treatment induces significant and multi-fold increase in proinflammatory cytokine secretion of IL-6, KC, and MCP-1, but there was no change in expression of IL-1E. TNF-D, RANTES and MIP-1D. Moreover, treatment of Aorta smooth muscle cells (AoSMCs) with different doses of nicotine did not induce expression of these pro-inflammatory cytokines either alone or in the presence of elastase-exposed cells. The researchers in vitro data shows that the secretion of IL-1E from RAW264.7 macrophages was significantly enhanced by concomitant nicotine treatment (10PM) with transient elastase compared to elastase treatment alone after 24hrs. 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 researchers recent results. The researchers findings also implicate the specific role of MDSC subsets and CXCR2 signaling that has the potential to influence aortic inflammation and vascular remodeling to cause AAA formation.

Follow on Funding: None at the time of reporting.

Collaborations: This research is being conducted in the Aortic Aneurysm Research laboratory at the Department of Surgery, University of Florida, Gainesville, FL. Trainee, Joseph Hartman,

(first year, medical student, University of Florida), and Jonathan Krebs, MD, (postdoctoral fellow) are performing research under this project.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

3. **Grant#:** 20K07 Molecular mechanisms and pharmacologic targeting of lipogenesis in breast cancer.

Principal Investigator: Daiqing Liao, PhD

Organization: University of Florida

Summary: Researchers aim to define molecular interactions between death doman-associated protein (DAXX) and sterol regulatory element binding protein 2 (SREBP1/2) and determine roles of SREBP1/2 and DAXX's small ubiquitin-like modifiers (SUMO) binding property in DAXX-driven oncogenic lipogenesis The researchers have conducted experiments to assess DAXX-SREBP1/2 interactions in cells co-transfected with DAXX and mature forms of SREBP1/2. The researchers observed that WT DAXX seemed to stabilize the mature form of SREBP1 but destabilize the mature SREBP2. Mutations (Y59/Y89F and Y124/Y126F) within the DAXX 4HB domain that is implicated with binding to SREBP1/2 in the researchers recently published data (PIMD: 37045819) reversed this effect. These data suggest that the DAXX SREBP1/2 interactions may regulate the protein stability of SREBP1/2, providing a potentially new mechanism by which DAXX regulates lipogenesis. Researchers aim to determine the role of mammalian target of rapamycin complex 1 (mTORC1) in DAXX S671 phosphorylation and the functional impact of the mTORC1-DAXX axis on the DAXX-SREBP1/2 interactions, lipogenesis and tumor growth. As reported previously, mTORC1 was not the kinase that phosphorylates DAXX at S671. The researchers have conducted studies to identify potential DAXX S671 kinases. Based on the site score system available in the PhosphoSitePlus® database, P38D (p38 delta, encoded by mitogen-activated protein kinase 13 (MAPK13)) is the top-ranked kinase for DAXX S671. The researchers have depleted MAPK13 using small interfacing ribonucleic acid (siRNAs). Western blotting experiments showed that MAKP13 knockdown significantly reduced the level of phosphorylated DAXX at S671, suggesting that the MAP kinase P38D is a likely candidate that specifically phosphorylates DAXX at S671. The data provides important information for understanding the signaling events that DAXX's biological function.Finally researchers will evaluate mechanism of action, in vivo safety, pharmaceutical properties and efficacy of the DAXX single-minded homolog 2 (SIM2) peptide on suppressing lipogenesis and breast tumor growth.

Follow on Funding: None at the time of reporting.

Collaborations: Nikee Awasthee: PhD, postdoctoral associate (Daiqing Liao, Mentor), University of Florida; Chengcheng Meng: MS graduate student (Daiqing Liao, mentor), University of Florida; Seth Hale: PhD, graduate student (Daiqing Liao, mentor), University of Florida; Aaron Chait: MS graduate student (Daiqing Liao, mentor), University of Florida; Brandon Kim: MS graduate student (Daiqing Liao, mentor), University of Florida.

Journals: Mahmud I, Tian G, Wang J, et al. DAXX drives de novo lipogenesis and contributes to tumorigenesis. *Nat Commun*. 14, 1927 (2023). Doi:10.1038/s41467-023-37501-0.

Patents: None at the time of reporting.

4. **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

Summary: Thirty-five community-dwelling individuals post-stroke have been enrollled in this randomized controlled trial. As the trial is ongoing research staff 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, which will not be complete until the completion of the study.

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. The University of Florida /College of Health and Human Performance/Departent 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 and 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.

5. **Grant#:** 20K09 Nicotine alters brain metabolism and exacerbates ischemic brain damage

Principal Investigator: Ami Raval, PhD, MSPH

Organization: University of Miami

Summary: Additional progress has been made in the proposed experiments investigating the duration for which nicotine (N) and oral contraceptive (OC) (N+OC)-induced harmful effects on mitochondria functions persist after N+OC withdrawal. Thus far analysis of the data suggests that possibly N+/-OC exposure's influence on mitochondrial protein levels persists after 30 days of withdrawal (W). Once remaining samples are assessed, statistical analysis will be carried out

to determine any statistically significant changes. Also, researchers have detailed the observations of changes in fat and phosphatidylcholine metabolism in brain tissue of N+OC exposed rats for identification of metabolic signatures. To identify if these changes may be persisting after withdrawal, analysis was performed to assess steady state levels of enzymes responsible for mediating aspects of fatty acid and phosphatidylcholine metabolism. Once the remaining samples have been analyzed, statistical analysis will be carried out to assess if any statistically significant changes exist. Researchers had determined that significantly higher infarction as well as hippocampal dependent memory loss was present in nicotine versus saline treated rats that underwent transient middle cerebral artery occlusion (tMCAO) 0 and 15 days after withdrawal. Subsequently, the research team began assessment of neuronal death in the CA1 region of the hippocampus of these exposed rats. These slides were analyzed in a stereoscopic manner with the use of Neurolucida (MBF neuroscience) program, and estimated cell counts for this region were calculated.

Follow on Funding: None at the time of reporting.

Collaborations: Mr. Sebastian Vargas-George is an undergraduate Neuroscience student working on a senior's honors thesis intended to be submitted this spring 2023. Ms. Gina Perez is an undergraduate Neuroscience student working on a senior's honors thesis on intended to be submitted this spring 2023. Ms. Khushi Shah is an undergraduate Neuroscience student working on a senior's honors thesis intended to be submitted this spring 2023.

Journals: Pradhyumnan, H, Reddy, V, Bassett, ZQ, et al. Post-stroke periodic estrogen receptor-beta agonist improves cognition in aged female rats. *Neurochemistry International*. 165(2023), 105521. Doi:10.1016/j.neuint.2023.105521.

López-Morales MA, Escobar I, Saul I, et al. Resveratrol Preconditioning Mitigates Ischemia-Induced Septal Cholinergic Cell Loss and Memory Impairments. *Stroke*. 2023;54(4):1099-1109. Doi:10.1161/STROKEAHA.122.040899.

Patents: None at the time of reporting.

6. **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

Summary: The research team has been working on two manuscripts from the results of the focus group study. Thus far, the research team has completed the two manuscripts' analysis and started the writing. Florida International University (FIU) obtained institutional review board (IRB) approval, hired and trained study personnel, and received approval to remove the plasma nicotine assessment from the battery of measurements done in the lab study in preparation for the next stage of the project. Participant enrollment began in December 2022 under the direction of Dr. Wasim Maziak. Thus far, 18 participants have been enrolled in the lab study. The research team requested an no-cost amendment to conduct a satellite study examining the effect of waterpipe smoking on cardiopulmonary exercise capabilities among young waterpipe smokers which will allow the researchers to develop even more age specific HWLs for young people. To disseminate knowledge on the research, Dr. Asfar provided an oral presentation

about the project at the World Health Organization (WHO) Fourth International Congress on Waterpipe Tobacco Smoking on September 27-29. Dr. Asfar's research team also presented a poster presentation about the project at the December 2-3, 2022, Sylvester Comprehensive Cancer Center Annual Retreat titled, "National Experimental Study Testing the Effectiveness of Pictorial Waterpipe-Specific Health Warning Labels Among Young Adults in the USA." The poster presentation focused on firstly, the online results of comparing the effect of the pictorial HWLs to the FDA health warning labels, and secondly on comparing the effect of the pictorial HWLs between six themes (waterpipe addiction, waterpipe harm compared to cigarettes, waterpipe harm to others, waterpipe health effects, waterpipe quitting, and waterpipe specific harms). Researchers continue to add information about the study and relevant publications to the website: (https://www.publichealth.med.miami.edu/research/research-labs/hookah-and-e-cigarettes-health-communication-group/index.html). Researchers also continue to meet regularly with the research consultant Mr. Abrams and colleagues in Golin to create new content for the website and social media accounts. The Golin team continues to help disseminate the overall study progress, focus group results, and final warning labels being tested in the lab setting by developing social media assets that can be shared across various platforms.

Follow on Funding: None at the time of reporting.

Collaborations: Florida International University: The researchers continue to meet bimonthly with colleagues at Florida International University (FIU) according to the executed agreement to discuss study procedures, recruitment methods and progress, and the project website. University of Memphis: Dr. Michael Schmidt, a graphic design expert, has completed the qualitative data analyses from all the focus groups. Truth Initiative: The researchers have continued a collaboration with consultants at the Truth Initiative, keeping them up to date on the study's progress. The research team created a modification to this agreement to replace Donna Vallone with Elizabeth Hair, PhD, Senior Vice President, as the main point of contact for the project.

Journals: Asfar T, Jebai R, Li W, et al. Risk and safety profile of electronic nicotine delivery systems (ENDS): an umbrella review to inform ENDS health communication strategies [published online ahead of print, 2022 Sep 8]. *Tob Control*. 2022;tobaccocontrol-2022-057495. Doi:10.1136/tc-2022-057495.

Schmidt, M, Asfar, T, Maziak, W. Graphic Design in Public Health Research. *Visible Language*. Vol. 56 No. 2 (2022): August 2022. https://journals.uc.edu/index.php/vl/article/view/6061.

Patents: None at the time of reporting.

Appendix M: James and Esther King Biomedical Research
Fiscal Year 2022-2023 Active Grants
Funded Fiscal Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
9JK03	Baptist Health South Florida	John Diaz, MD	$700,000.00	12/31/24	No	No	No

7. **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

Summary: The study KEYNOTE 826, a randomized phase III trial evaluating the addition of pembrolizumab to the chemotherapy in the management of recurrent or metastatic cervical cancer, demonstrated an improvement in progression-free and overall survival. Based on these results immunotherapy has been moved to the upfront treatment of metastatic cervical cancer. Researchers expect that these results will greatly reduce the number of pembrolizumab-naïve patients at time of recurrence who would be eligible for this trial. This will have a negative impact on enrollment and the ability to reach the desired number of patients. The research team continues to recruit patients for enrollment. To date, researchers have screened 12 patients and have successfully enrolled eight patients. The research team currently has three patients on trial who have responded based on recent imaging. Unfortunately, the researchers have not enrolled any additional patients during this reporting period.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix N: James and Esther Kin g Biomedical Research
Fiscal Year 2022-2023 Closed Grants
Funded Fiscal Year 2021-2022

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
22K04	University of Florida	Brian Law, PhD	$98,940.00	3/31/23	Yes	Yes	Yes
22K09	University of South Florida	Mohapatra Subhra, PhD	$100,000.00	3/31/23	No	No	No

1. **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

Summary: The purpose of this grant award was to perform studies that facilitate the translation of a new class of anticancer agents toward clinical use. These compounds, termed Disulfide bond Disrupting Agents (DDAs), exhibit a unique mechanism of action whereby they inactivate cancer cell receptors responsible for proliferation and survival and activate cancer cell receptors that promote cell death. These DDA effects are caused by inhibition of three enzymes responsible for the maturation of these receptor molecules. Importantly, DDA compounds are the only known compounds that inhibit two of these three enzymes, anterior gradient protein 2 homolog (AGR2) and endoplasmic reticulum-resident protein 44 (Erp44). This project provided new molecular details into how the DDA compounds selectively kill breast cancer cells without affecting normal cells or tissues. Further, DDA metabolism studies were carried out to identify DDAs that are not metabolized by human liver or intestinal enzymes and thus may be effective against breast tumors in clinical trials. This work identified a specific DDA compound, difluoro-tcy (dFtcyDTDO), that has strong anticancer activity against human breast tumors in experimental models and does not exhibit detectable metabolism by human liver or intestinal enzymes. Thus, the dFtcyDTDO compound is expected to have a long half-life and may be suitable for initiating clinical trials to determine whether dFtcDTDO or similar DDAs will induce breast tumor regression in patients and improve patient survival and quality of life.

Follow on Funding: Law, B, PI. NIH/NCI R21. 07/01/2023-06/30/2025. Total Funds Requested: $410,040. Pending.

Collaborations: The DDA compounds under investigation here are the only known inhibitors of the protein disulfide isomerases PDIs AGR2 and Erp44. For this reason, the research staff were contacted by two world leaders in Erp44 research, Dr. Roberto Sitia at Vita-Salute San Raffaele University, Milano, Italy and Dr. Boaz Tirosh at Case Western Reserve University, Cleveland, OH. Researchers have begun collaborations with both of these investigators and have provided DDAs to them and exchanged research materials (genetically altered cell lines) among the laboratories. It is expected that these collaborations will lead to additional future publications, grant applications, and discoveries. In fact there is already have a pending R01 application with Dr. Tirosh.

Journals: Ghilardi AF, Yaaghubi E, Ferreira RB, et al. Anticancer Agents Derived from Cyclic Thiosulfonates: Structure-Reactivity and Structure-Activity Relationships. *ChemMedChem*. 2022;17(14):e202200165. Doi:10.1002/cmdc.202200165.

Law ME, Yaaghubi E, Ghilardi AF, et al. Inhibitors of Erp44, PDIA1, and AGR2 induce disulfide-mediated oligomerization of Death Receptors 4 and 5 and cancer cell death. *Cancer Lett*. 2022;534:215604. Doi:10.1016/j.canlet.2022.215604.

Patents: Law, BK, Castellano, R, Ferreira, R, inventors. NOVEL SMALL MOLECULE ANTICANCER AGENTS. US patent 10,813,904. October 27, 2020.

Law, BK, Jahn, S, et al, inventors; University of Florida Research Foundation, assignee. NOVEL SMALL MOLECULE ANTICANCER AGENTS, COMBINATIONS AND USES THEREOF. US National Phase Application No.: 17/912,477, September 16, 2022; International Filing Date: March 16, 2021; Publication Date: April 20, 2023. Pending.

Law, BK, Castellano, R, inventors; University of Florida Research Foundation, assignee. INHIBITION OF THE PDI FAMILY MEMBERS AGR2, PDIA1, AND ERP44 FOR THERAPEUTIC TREATMENT AND USE IN PREDICTIVE DIAGNOSTICS/MONITORING FOR TREATMENT REGIMENS. US National Phase Application Nos.: PCT/US2022/011961 – WGS Ref.; No.: U1195.70190WO00; International Filing Date: January 11, 2022. Pending.

Law, BK, Castellano, R, inventors; University of Florida Research Foundation, assignee. ANTICANCER COMPOUNDS AND USES THEREOF. US National Phase Application No.: US 63/135,979; Filing Date: January 11, 2022. Pending.

Law, BK, Jahn, S, et al, inventors; University of Florida Research Foundation, assignee. NOVEL SMALL MOLECULE ANTICANCER AGENTS, COMBINATIONS AND USES THEREOF. US National Phase Application No.: US 62/990,544; Filing Date: March 17, 2020. Pending.

Law, BK, Jahn, S, et al, inventors; University of Florida Research Foundation, assignee. NOVEL SMALL MOLECULE ANTICANCER AGENTS, COMBINATIONS AND USES THEREOF. US National Phase Application No.: U1195, 70134US01; Filing Date: June 15, 2018. Pending.

2. **Grant#:** 22K09 Mechanism of neurotropism by coronaviruses

Principal Investigator: Mohapatra Subhra, PhD

Organization: University of South Florida

Summary: 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 severe acute respiratory syndrome coronavirus 2 (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 they can be directly infected, and molecular machinery responsible for neuroinvasion. The research team found that SARS-CoV-2 is detected in all cells by quantitative polymerase chain reaction (qPCR). Angiotensin-converting enzyme 2 (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 researchers are 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.

Appendix O: James and Esther King Biomedical Research
Fiscal Year 2022-2023 Closed Grants
Funded Fiscal Year 2019-2020

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
20K01	Florida State University	Pradeep G. Bhide, PhD	$626,708.00	5/31/23	No	No	No
20K02	Mayo Clinic Jacksonville	Debabrata Mukhopadhyay, PhD	$626,708.00	4/30/23	No	No	Yes
20K04	University of Central Florida	Ulas Bagci, PhD	$1,112,880.00	4/30/23	No	No	Yes
20K11	University of Miami	Miguel Perez-Pinzon, PhD	$626,708.00	5/31/23	No	No	No

1. **Grant#:** 20K01 Nicotine, germ cells and neurodevelopmental disorders

Principal Investigator: Pradeep G. Bhide, PhD

Organization: Florida State University

Summary: 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. During this reporting period, the research team continued studying the effects of exposure of pregnant mice to e-cigarette aerosol, e-liquid aerosol, or room air (control). Researchers focused on the development of neurons containing the neurotransmitter gamma aminobutyric acid (GABA). GABA is the principal inhibitory neurotransmitter in the central nervous system. Compromised GABA neurotransmitter system is a shared feature of neurodevelopmental disorders including attention deficit hyperactivity disorder (ADHD) and autism that are associated with exposure of the developing brain to nicotine. In this study, researchers used a transgenic mouse model that is genetically engineered such that all GABA neurons in the brains of the mice express a molecule called the green fluorescent protein. The fluorescence facilitates tracking the GABA neurons in the brain of embryos under a microscope. Researchers exposed pregnant transgenic mice to e-cigarette aerosol, e-liquid aerosol or room air beginning two to three weeks prior to conception until the 14th day of pregnancy during pregnancy until the day the embryos were collected for analysis. Researchers collected embryos on the 15th day of pregnancy, which represents the final third of the mouse pregnancy and is equivalent to the late second trimester of human pregnancy. Moreover, a critical event in the life history of the of GABA neurons is ongoing. It is migration of GABA neurons from the site of origin to their destinations in the cerebral cortex, which is the brain region that controls higher cognitive function. The migration process is vulnerable to the influences of deleterious substances such as nicotine and e-cigarette aerosol. The data showed that e-cigarette aerosol exposure produced deficits in GABA neuron numbers in the developing cerebral cortex offering the first and early evidence of compromised maturation of the GABA neurotransmitter system. In summary, this data shows that e-cigarette use during pregnancy is associated with changes in the development of the GABA neurotransmitter system of the brain of the growing fetus, which may presage deficits in neurotransmitter function of the mature brain.

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#:** 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

Summary: The research staff developed a proprietary multi-peptide-based vaccine formulation comprising multiple tumor-associated antigens (TAA) that are overexpressed in Non-Small Cell Lung Cancer (NSCLC) and evaluated its efficacy in a therapeutic setting in a mouse syngeneic model of lung cancer. Any additive/synergistic effect of the vaccine on chemotherapy or immunotherapy were also assessed. Briefly, a subcutaneous model was developed in C57BL6 mice by implanting 1 X 10^5 LLC1 cells into the right flanks. Tumors were allowed to grow for 12 days, and then tumor-bearing mice were randomized in six treatment groups as follows: i) Untreated control, ii) Vaccine, iii) Anti-programmed cell death protein (PD-1) antibody, iv) Vaccine + Anti-PD-1 antibody, v) Pemetrexed, and vi) Vaccine + Pemetrexed. Three doses of vaccine (four peptides x 100 ug per peptide for each dose) were administered intradermally per mouse one week apart. Anti-PD-1 antibody (200 ug/mouse) and pemetrexed (10 mg/kg) were given two times a week for three weeks intraperitoneally. The untreated tumors became ulcerated after two weeks and lost tumor mass leading to seemingly lower endpoint tumor volume. Anti-PD-1 antibody could not inhibit tumor growth. The vaccine itself was able to delay tumor growth to some extent. However, the most noticeable tumor growth inhibition was observed in the vaccine + anti-Pd-1 antibody treated group. The combination of vaccine with pemetrexed didn't provide any significant benefit over the vaccine-treated group. Mice were sacrificed after three weeks of treatment, and tumors were harvested for future immunohistochemical analysis to determine any alterations in tumor-infiltrating immune cell populations. These studies are in progress. The research staff harvested spleens from the mice in the above experiment and prepared single cell suspensions of splenocytes following standard procedure. Pemetrexed and Vaccine + pemetrexed groups were not included in this experiment since no significant benefit were observed in this combination. The released Lactate dehydrogenase (LDH) levels were measured following the manufacturers protocol (Promega, USA cat#G1780). Consistent with the in vivo tumor growth inhibition data, the combination group showed the most target cell lysis.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Pal K, Hussain T, Xie H, et al. Expression, correlation, and prognostic significance of different nicotinic acetylcholine receptors, programed death ligand 1, and dopamine receptor D2 in lung adenocarcinoma. *Front Oncol*. 2022;12:959500. Published 2022 Aug 22. Doi:10.3389/fonc.2022.959500.

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

Summary: The overall goal of this project was to develop and test cutting edge machine learning models targeted to clinicians when treating lung cancer patients with SBRT (stereotactic body radiotherapy). Currently, if cancer regions are not optimized during radiotherapy treatment for radiation dose delivery and local regrowth occurs, this regrowth is hard to identify in medical images until much later. Similarly, predicting if the tumor is responsive to the therapy or not at earlier stages will give clinicians chances to optimize the therapy regimes, or seek alternatives. The proposed tool can assist with accessing the risk of individual patients based on their imagery alone or at any point along a patient's treatment process, which is novel compared to what is currently available.The project developed a novel method to predict overall survivability of patients based only on their planning computed tomography (CT) before treatment. The effectiveness of the tool was assessed using Kaplan-Meyer single factor survival analysis and Cox Proportional multi-factor analysis. The analysis result is that this tool was considered a strong predictor when compared with traditional clinical metrics currently used to access survival risk. The project also pioneered the combination of radiomics measurements taken from images with deep learning classification networks to provide improved accuracy predicting the overall survivability when compared with either of the methods alone. Furthermore, researchers developed a novel, cloud-based image annotation system for clinicians to provide temporal annotations about the treatment trajectory of patients. This interface will provide annotations useful in training temporal treatment response machine learning models. This research developed and conducted early validation of the value of image-based machine learning methods to create treatment planning tools. The research teams believe the results are very promising and there is a clear path to add deep learning to the existing explainable artificial intelligence (AI) tools developed during this program. For next steps, the research team plans to expand to take advantage of the temporal information contained in the cohort of SBRT patients by using an annotation interface developed during this period to create training data for temporally predictive AI models. Researchers plan to apply for National Institutes of Health (NIH) research funding after further refinement of the process.

Follow on Funding: None at the time of reporting.

Collaborations: This was the first funded research collaboration between the University of Central Florida (UCF) and Orlando Health, the only Level 1 Trauma Center in the Central Florida area. This project was the first opportunity for KnowledgeVis, LLC to work directly with Orlando Health Cancer Center clinicians to get feedback on how these research tools can be used directly in clinical applications to aid the treatment of Floridians with lung cancer. This project involved student contributors from both UCF's Computer Science department (a doctoral student) and the UCF Medical School (a medical student) engaging with practicing clinicals from Orlando Health Cancer Center. This research strengthened the relationships between the research conducted at UCF and the clinical care offered to directly benefit Floridians at Orlando Health. Each organization was excited to participate on this project.

Journals: Isler, I, Lisle, C, Rineer, J, et al. Enhancing organ at risk segmentation with improved deep learning networks. *Proceedings of the SPIE*, Volume 12032, id. 1203233 7 pp. (2022). Doi:10.1117/12.2611498.

Isler, I, Jha, D, Lisle, C, et al. Self-Supervised Learning for Organs At Risk and Tumor Segmentation with Uncertainty Quantification. Eprint arXiv:2305.02491 (2023) doi: 10.48550/arXiv.2305.02491.

Patents: None at the time of reporting.

4. **Grant#:** 20K11 Strategies to ameliorate cognitive decline following cerebral ischemia in nicotine-exposed rats

Principal Investigator: Miguel Perez-Pinzon, PhD

Organization: University of Miami

Summary: Over the course of the three-year grant period, extensive studies have provided significant insights into the impact of physical exercise (PE) and resveratrol (RSV) on cognitive outcomes post-Middle Cerebral Artery Occlusion (MCAo). The research concluded that PE appears to enhance neuronal survival and reduce cognitive deficits post-MCAo, potentially through an epigenetic and transcriptional reprogramming that boosts brain plasticity. The data also indicated that ischemia led to a significant reduction in gamma aminobutyric acid (GABA)ergic cells, while cholinergic cell counts remained unaffected. When RSV was introduced into the mix in the third year, the research team observed that it helped mitigate memory and functional impairments in the hippocampus caused by stroke, by significantly reducing the loss of cholinergic cells within the medial septal nucleus and diagonal band of Broca. Although the preliminary data on the combined effects of PE and RSV were inconclusive, these studies provide strong evidence to support further exploration into their potential synergistic impacts on cognitive recovery post-MCAo. Research staff will be consolidating all the data collected from this project and expect to have it published. This project has also allowed the team to gather enough data to propose a follow up grant application that the research team expects to submit in few months.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix P: James and Esther King Biomedical Research
Fiscal Year 2022-2023 Closed Grants
Funded Fiscal Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
9JK01	Florida State University	Gloria Salazar, PhD	$805,409.00	3/31/23	Yes	No	Yes
9JK02	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Shelley Tworoger, PhD	$504,838.00	8/31/22	Yes	No	Yes
9JK04	University of Central Florida	Alicja Copik, PhD	$805,409.00	3/31/23	Yes	Yes	Yes
9JK05	University of Florida	Ramzi Salloum, PhD	$404,909.00	3/31/23	Yes	Yes	Yes
9JK06	University of Florida	Maria Zajac-Kaye, PhD	$805,409.00	3/31/23	No	Yes	Yes
9JK07	University of Miami	Sundaram Ramakrishnan, PhD	$805,393.00	2/28/23	No	No	Yes
9JK08	University of Miami	Kunjan Dave, PhD	$805,409.00	3/31/23	Yes	No	Yes
9JK09	University of Miami	Nipun Merchant, MD	$805,409.00	3/31/23	No	No	Yes
9JK10	University of South Florida	Rex Philpot, PhD	$771,341.00	7/31/22	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

Summary: The research team evaluated sex-dependent differences in atherosclerosis in apolipoprotein E (ApoE)-/- mice. Research staff exposed male and female ApoE-/- mice to cigarette smoke (CS) and nicotine for four months and measured body weight, and food and water intake. Male and female mice exposed to nicotine ate more food across all weeks than control and CS groups. Females showed a tendency toward higher food intake that was significant only in some weeks. For water intake, males in the CS group drank more all weeks. In contrast, females in this group drank more water than the nicotine. Nicotine groups drank less water only in the first few weeks of treatment. Their water intake remained similar to controls for the rest of the experiment. Compared to week one, body weight increased in all groups. For body composition, compared with week one, males in all groups gained fat, and lost lean mass and total water by week 16. Females showed no differences in fat or lean mass, but controls had increased total water at week 16. Baseline body composition characteristics (week one) between sexes differed, as females had more fat mass and lower lean and water masses. However, at week 16, females had less fat and more water in the CS group and more water in the control group. Compared to control, plaque was higher in the arch of nicotine and CS exposed mice in both sexes, but only females accrued more plaque in the descending aorta by both treatments. Like plaque content, senescence-associated galactosidase, beta 1 (GLB1)/β-galactosidase (SA-GLB1) activity was upregulated in all groups and was higher in females in the CS. In males, CS increased total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL), compared with control and nicotine. No changes were seen in low-density lipoprotein (LDL), while glucose was increased by CS and nicotine. The liver enzymes aspartate and alanine aminotransferases (AST, ALT) were elevated by CS, but only AST reached significance compared to control. Estradiol was higher in the nicotine and CS groups, while testosterone was not affected by any treatment. In contrast, in females CS increased total cholesterol (TC), LDL and aspartate aminotransferase (AST),

Estradiol was reduced by smoking, but was not different from control. Progesterone was reduced by CS. Thus, reduced estradiol, which protects females from CVD, is likely not the cause of increased plaque by CS or nicotine. Overall, plaque and lipid profile differed between sexes, as females displayed greater plaque in the control and CS groups and a trend towards more plaque by nicotine. These differences were not correlated with more circulating lipids. In fact, females had less TC, TG, HDL and VLDL than males in all groups and lower glucose in the nicotine and CS groups. Although LDL was elevated by CS in females, its levels were not different between sexes in any groups.

Follow on Funding: Salazar, G, PI, Remodeling of the microbiome by E-cigarette vapors and its effects in atherosclerosis. NIH R01 12/1/2023-11/30/2028. Total Amount Requested: $3,124,309. Submission Date: 03/05/2023. Pending.

Muller-Delp, J, PI, Salazar, G, Co-PI, Role of Adiponectin in Reversal of Age-related Vascular Dysfunction. NIH R01 02/15/2023-11/30/2026. Total Amount Awarded: $1,895,484. Submission Date: 06/05/2022.

Parvatiyar, M, PI, Salazar, G, Co-PI, Determining How Tobacco Use and Obesity Exacerbates a Novel Cardiovascular Risk Factor. FDOH James and Esther King Biomedical Research Program. 04/01/2021-5/31/2024. Total Amount Awarded: $535,396. Submission Date: 09/08/2020.

Pradeep, B, PI, Salazar, G, Collaborator, Nicotine, germ cells and neurodevelopmental disorders. FDOH James and Esther King Biomedical Research Program. 06/01/2020-05/31/2023. Total Amount Awarded: $626,708. Submission Date: 09/08/2020.

Collaborations: None at the time of reporting.

Journals: Serino A, Zhao Y, Hwang J, et al. Gender differences in the effect of blackberry supplementation in vascular senescence and atherosclerosis in ApoE-/- mice. J Nutr Biochem. 2020;80:108375. doi:10.1016/j.jnutbio.2020.108375.

Centner AM, Bhide PG, Salazar G. Nicotine in Senescence and Atherosclerosis. Cells. 2020;9(4):1035. Published 2020 Apr 22. doi:10.3390/cells9041035.

Cullen AE, Centner AM, Deitado R, Salazar JFA. The Impact of Dietary Supplementation of Whole Foods and Polyphenols on Atherosclerosis. Nutrients. 2020;12(7):2069. Published 2020 Jul 12. doi:10.3390/nu12072069.

Klionsky DJ, Abdel-Aziz AK, Abdelfatah S, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition)1. Autophagy. 2021;17(1):1-382. doi:10.1080/15548627.2020.1797280.

Clark M, Centner AM, Ukhanov V, Nagpal R, Salazar G. Gallic acid ameliorates atherosclerosis and vascular senescence and remodels the microbiome in a sex-dependent manner in ApoE-/-mice. J Nutr Biochem. 2022;110:109132. doi:10.1016/j.jnutbio.2022.109132.

Khalili L, Centner AM, Salazar G. Effects of Berries, Phytochemicals, and Probiotics on Atherosclerosis through Gut Microbiota Modification: A Meta-Analysis of Animal Studies. Int J Mol Sci. 2023;24(4):3084. Published 2023 Feb 4. doi:10.3390/ijms24043084

Centner AM, Khalili L, Ukhanov V, Kadyan S, Nagpal R, Salazar G. The Role of Phytochemicals and Gut Microbiome in Atherosclerosis in Preclinical Mouse Models. Nutrients. 2023;15(5):1212. Published 2023 Feb 28. doi:10.3390/nu15051212.

Cullen, A, Centner, A, Deitado, R, et al. The Duality of Adiponectin and the Role of Sex in Atherosclerosis. JCI Insights. bioRxiv PrePrint. doi:10.1101/2023.05.23.541764. Pending.

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, Inc.

Summary: The accomplishments included developing a detailed analysis plan for each study aim and examining the distributions of the primary exposures in the Nurses' Health Study (NHS) and NHSII. In addition, for secondary analyses of the early life exposures and ovarian tumor immune response, tissue microarrays (TMAs) containing ovarian tumor tissue from cases in the NHS and NHSII were used. The research completed preliminary analyses of early life smoking exposure and early life abuse with ovarian cancer risk. In the smoking analysis, the results showed no association of age at smoking initiation with risk; however, women with a parent who smoked while pregnant or in the home had a modest, statistically significant increased risk of ovarian cancer. In the abuse analysis, which was limited to NHSII since abuse questions where not asked in NHS, there was a suggestive, but non-statistically significant, increased risk of ovarian cancer among women who had experienced higher levels of physical and emotional or sexual abuse as a child or teenager. Analysis results showed that having a parent who smoked in the home was primarily associated with risk of developing non-serous or low-grade serous tumors; no association was observed with risk of high-grade serous tumors, the most common ovarian cancer subtype. Associations were similar among women who did and did not become smokers themselves in adulthood. Research staff conducted preliminary bivariate analyses examining the association of each early life exposure with tumor immune cells, including T cells, B cells, and tumorassociated macrophages (TAM). These analyses suggested that participants whose parents were farmers, did not own their home, or had a high school education or less had lower levels of tumor-fighting helper and cytotoxic T cells as well as a lower levels of antibody-producing plasma cells in their tumors. Also, higher levels of early life abuse were associated with lower intratumoral T cells and B cells. The Black Women's Health Study and Sister Study data were added to the study to increase life abuse and ovarian cancer risk sample size. No clear association of ovarian cancer risk was found with physical activity at ages 12-13, 14-17, or 18-22 or average physical activity across the three periods. Analyses of early life abuse (Aim 2) were finalized. Overall, no associations of ovarian cancer risk with early life sexual or physical abuse were observed in any of the studies. Research staff finalized analyses of lifetime smoking exposure and ovarian cancer risk by T cells in the tumor; results showed that women exposed to cigarette smoke early in life had a higher risk of developing ovarian cancer with low levels of T cells overall and low levels recently activated cytotoxic T cells. Analyses of early life (premenopausal) physical activity and ovarian cancer risk by tumor immune markers (Aim 3) were completed. Research staff found similar results across T cells and B cells such that women who were highly active during their premenopausal years tended to have an increased risk of developing tumors with low immune cell abundance compared with less active women.

Follow on Funding: Kubzansky, L, PI. Department of Defense, Office of the Congressionally Directed Medical Research. 05/01/2021-04/30/2024. National Institutes for Health/National Cancer Institutes.

Collaborations: This grant fostered the initiation of a collaboration between Dr. Jake-Schoffman, of the University of Florida, with the rest of the research team based at Moffitt Cancer Center. Dr. Jake-Schoffman's scientific expertise, particularly in the area of physical activity, was critical for the success of the grant and will remain involved in completing manuscripts related to physical activity after the end of the award.

Journals: Hathaway CA, Wang T, Townsend MK, et al. Lifetime Exposure to Cigarette Smoke and Risk of Ovarian Cancer by T-cell Tumor Immune Infiltration. *Cancer Epidemiol Biomarkers Prev*. 2023;32(1):66-73. doi:10.1158/1055-9965.EPI-22-0877.

Patents: None at the time of reporting.

3. **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

Summary: 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 lung cancer, the response rate is still low, and most patients eventually relapse. The goal of this project was to develop clinically translatable approaches for lung cancer treatment to increase the response rate to approved or upcoming checkpoint inhibitor therapies. To achieve this, the project leveraged the unique membrane bound interleukin-21 (PM21)-technology developed in the laboratory to multiply to therapeutically relevant numbers and activate natural killer (NK) cells. In this project it was also shown that PM21-NK cells maintain function after thawing, an aspect critical for their development as a cellular therapeutic (Front. Immun. 2022). Methods were also developed to engineer NK cells to improve their function and make them compatible with immunotherapies such as anti-PD-L1 and the highly promising anti- T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT). Anti-TIGIT therapeutics have generated great excitement based on early clinical results but phase III trials in patients with lung cancer, so far have been disappointing. This project demonstrated TIGIT is highly expressed on functional NK cells (Cancers 2023) and that NK cells are critical for efficacy of immunotherapies (Review in Front. Immun. 2021). It was also shown that current anti-TIGIT therapeutics tested can potentially deplete critically important NK cells in treated patients (Under submission to Journal for ImmunoTherapy of Cancer (JITC) and patented). This could negatively impact treatment outcomes. As potential solution, gene editing was used to delete (knockout, KO) the receptor. TIGIT KO in NK cells prevented NK cell depletion by anti-TIGIT therapeutics. TIGIT KO NK cells were able to kill tumors better and were more metabolically fit. These modified TIGIT KO NK cells have the potential to be therapeutically more effective and improve the outcomes of the highly anticipated anti-TIGIT therapeutics. A patent from this work 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 programmed cell death ligand 1 (PD-L1). These modified NK cells kill tumors well and are also resistant to self-destruction when

combined with an immunotherapeutic antibody called Avelumab (patent and manuscript in prep). These modified NK cells will likely be undergoing clinical development and if approved will benefit Floridians.

Follow on Funding: Copik, A, PI. Florida Department of Health, Live Like Bella. 04/01/2022-03/31/2025, Funds Awarded: $250,000, Submission date 09/08/2021.

Collaborations: None at the time of reporting.

Journals: Shaver, KA, Croom-Perez, TJ, Copik, AJ. Natural Killer cells: the linchpin for successful cancer immunotherapy. *Front Immunol*. 2021; 12:679117. doi:10.3389/fimmu.2021.679117.

Oyer, JL, Croom-Perez, TJ, Dieffenthaller, TA, et al. 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.

Croom-Perez, TJ, Robles-Carillo, LD, Dieffenthaller, TA, et al. Kinetic, Imaging Based Assay to Measure NK Cell Cytotoxicity Against Adherent Cells. *Methods Cell Biol*. 2023;178:63-91. doi:10.1016/bs.mcb.2022.07.012.

Hasan, MD, Croom-Perez, TJ, Oyer, JL, et al. TIGIT Expression on Activated NK Cells Correlates with Greater Anti-Tumor Activity but Promotes Functional Decline upon Lung Cancer Exposure: Implications for Adoptive Cell Therapy and TIGIT-Targeted Therapies. *Cancers*. 2023; 15(10):2712. https://doi.org/10.3390/cancers15102712.

Patents:

Copik AJ, Hasan MF, Croom-Perez TJ, inventors. ENGINEERED NK CELLS AND USES THEREOF-provisional patent application was filed on 09/29/2021, PCT filed 09/29/2022. Pending.

Copik AJ, Croom-Perez TJ, Oyer JL, et al. COMBINATION THERAPY COMPRISING PD-L1 KNOCKOUT NK CELL AND ANTI-PDL1 ANTIBODIES-provisional patent application was filed on 04/08/2022, PCT filed 04/10/2023. Pending.

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

Principal Investigator: Ramzi Salloum, PhD.

Organization: University of Florida

Summary: The purpose of this grant was to evaluate the feasibility of reducing secondhand tobacco smoke exposure in children within the pediatric care setting. This project took 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.Accomplishments include MyChart activation at clinic sites, development of Bright Futures Questionnairs for the identified well-child visit age ranges in the MyChart platform, and utilization of IPads for questionnaire data collection from patients. Unsolicited patient feedback

to physicians revealed that some parents were pleasantly surprised to see the Bright Futures questionnaires in an electronic format. Physicians and residents also voiced appreciation to see the results of these questionnaires prior to physically seeing their patients. This could improve the efficiency of their visits and allow them to discuss topics that matter most to patients and their parents. A total of 5,065 completed questionnaires were collected. A total of 60 participants with tobacco use exposure were enrolled and 39 participants completed the three month follow up questionnaire.

Follow on Funding: Salloum, R, PI. Aetna Foundation. 03/01/2020-10/31/2022, Aetna Total Amount Awarded: $225,000.

Collaborations: The study is to be conducted entirely at the University of Florida (UF) 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. Dr. Salloum is an economist and implementation scientist in the Department of Health Outcomes and Biomedical Informatics in Gainesville. The Co-Investigators on this project are located at UF : Dr. Elizabeth Ann Shenkman is the Chair of the Department of Health Outcomes and Biomedical Informatics and also is the Co-Director of the University of Florida Clinical and Translational Science Institute (CTSI) and the Associate Director for Community Outreach and Engagement for the UF Health Cancer Center in Gainesville. Dr. Matthew J. Gurka is a Professor in the Department of Health Outcomes and Biomedical Informatics and the department's Associate Chair of Education at the University of Florida in Gainesville. Dr. Ryan P. Theis is an Assistant Professor in the Department of Health Outcomes and Biomedical Informatics in Gainesville. Dr. Jiang Bian is the Chief Data Scientist for UF Health, Director of Cancer Informatics Shared Resource and Associate Director of the Biomedical Informatics Program for the UF CTSI. Dr. Lindsay A. Thompson is a Professor of Pediatrics and Health Outcomes and Biomedical Informatics at the University of Florida in Gainesville..Research staff supported by this project are located at UF: Dr. Anna Maria Abi Nehme serves as the study coordinator and oversees all study activities.

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

LeLaurin JH, Nguyen OT, Thompson LA, Hall J, Bian J, Cho HD, Acharya R, Harle CA, Salloum RG. Disparities in Pediatric Patient Portal Activation and Feature Use. *JAMIA open*. 2021 Jul;4(3):ooab086.

Patents: None at the time of reporting.

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

Principal Investigator: Maria Zajac-Kaye, PhD

Organization: University of Florida

Summary: The goal of this grant was to demonstrate the antitumoral effect of a newly identified thymidylate synthase (TS) inhibitor compound P (Mefloquine) alone and in combination with Kirsten rat sarcoma (KRAS) effector pathway inhibitors in pancreatic ductal adenocarcinoma (PDAC). The University of Florida concluded that Mefloquine alone increases survival of

hTS/KrasG12DPten+/- genetically engineered animal models (GEMM) when administered at 100 mitogen-activated protein kinase (MPK) by oral gavage as compared to vehicle treated animals. Treatment of hTS/KrasG12D GEMMs with Mefloquine at 100 and 200 MPK also increased survival, however, it did not reach statistical significance and thus more animals will be needed to reach final conclusion. In addition, researchers found that a combination of 200 MPK Mefloquine + 5 MPK Everolimus or 100 MPK Mefloquine + 2.5 MPK Everolimus in both animal models, does not confer survival advantage compared to Mefloquine alone. This effect may be due to the high efficacy of Everolimus alone. Additional combination studies using 100 MPK Mefloquine with lower doses of Everolimus are required to establish synergy between both drugs in hTS/KrasG12D and hTS/KrasG12DPten+/- pancreatic ductal adenocarcinoma (PDAC) mouse models. The research team have also determined that two additional mammalian target of rapamycin (mTOR) inhibitors, Temsirolimus (rapalog inhibitor of mTOR) and TAK-228 (a highly selective mTORC1/TORC2 inhibitor): 1) have a potent cytotoxic effect alone in PDAC cell lines, and that 2) when combined with Mefloquine induce synergy in vitro. Researchers have also determined that Temsirolimus and Sapanisertib (TAK-228) alone have a potent antitumoral effect in Luc PANC-1 xenograft model. Future work to establish whether combination of 100 MPK Mefloquine with Temsirolimus and TAK-228 have synergistic effect in vivo. In addition, in this grant researchers have studied the antitumor activity of Mefloquine combined with KRAS effector pathway inhibitors in human samples using patient derived xenografts (PDX) from PDAC biopsies annotated for smoking status. The research team observed that 100 MPK Mefloquine reduces tumor volume in both female and male nonsmokers while tumor volume was reduced only in a female smoker. These are interesting results; however, more human samples need to be tested to reach final conclusions. 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: Guijarro MV, Kellish PC, Dib PE, et al. First-in-class multifunctional TYMS nonclassical antifolate inhibitor with potent in vivo activity that prolongs survival. JCI Insight. 2023;8(10):e158798. Published 2023 Apr 25. doi:10.1172/jci.insight.158798.

Vijayakurup V, Maeng K, Lee HS, et al. Thymidylate synthase accelerates Men1-mediated pancreatic tumor progression and reduces survival. JCI Insight. 2022;7(19):e147417. Published 2022 Oct 10. doi:10.1172/jci.insight.147417.

Dib, PE, Guijarro, M, Kellish, P, et al. Abstract 1657: Discovery of a first-in-class multifunctional TYMS non-classical antifolate inhibitors with potent in vivo activity that prolongs survival. Cancer Res. 1 April 2023; 83 (7Supp):1657. doi:10.1158/1538-7445.AM2023-1657.

Patents: Zajac-Kaye, M, Francois, R, Inventors; University of Florida, assignee. COMPOSITIONS FOR THE TREATMENT OF CANCER AND USES THEREOF. UF Ref. No.: T15748. U.S. Patent Application No.: 15/737,545. Approval date 07/06/2020.

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

Principal Investigator: Sundaram Ramakrishnan, PhD

Organization: University of Miami

Summary: Cigarette smoke increased pancreatic cancer growth. Depleting the gut microbiome by antibiotics abrogated the effect of smoking on cancer growth implying the role of gut microbiome in smoking-induced promotion of cancer growth. Cigarette smoke however failed to promote tumor growth in immunocompromised mice. Tobacco smoke component, 4-methylnitrosoamino-1(3-pyridyl)-1-butanone (NNK) exposure altered gut microbiome of mice. Transfer of microbiome from the NNK-treated mice into antibiotics treated recipient mice increased pancreatic cancer growth implying that changes in the microbiome mediates tumor progression. NNK-microbiome mediated changes in cancer growth is dependent on functional immune cells. Smoking induces hypoxia alters tumor microenvironment. Hypoxia-induced micro-ribonucleic acid (RNA)-210 when deleted was found to increase tumor growth. Pancreatic cancer was found to be highly sensitive to protein translation elongation inhibitor, homoharringtonine. The COVID19-related slow down delayed some of the in vivo experiments. Animal work has significantly improved since the beginning of the year. This will allow the research team to accelerate these studies to accomplish the stated goals of the project. These studies will include microbial metabolite analysis, immunotherapy modulation by NNK-induced microbial dysbiosis. Smoking and obesity are the major risk factors for pancreatic cancer development. Smoke-induced changes in microbiome and its role in cancer growth has been established in these studies. Currently, obesity (high-fat diet) either alone or in combination with cigarette smoke is studied for gut microbial dysbiosis and its impact on pancreatic cancer growth. Ongoing studies (current and No-Cost Extension period) focused on characterizing microbiome and metabolite changes induced by cigarette smoke alone and obesity. These studies are progressing and will be completed in the near future (metabolite characterization). Fecal microbial transplant (FMT) between control and dysbiotic microbiome will be used to investigate the impact of gut-immune axis alterations by smoking and high-fat diet. These studies will be followed with the effect of immune check point inhibitors and characterize transcriptomic changes in the tumor microenvironment. Data generated from these studies will be used in submitting an NIH application.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Ma S, Mangala LS, Hu W, et al. CD63-mediated cloaking of VEGF in small extracellular vesicles contributes to anti-VEGF therapy resistance. *Cell Rep*. 2021 Aug 17;36(7):109549. doi:10.1016/j.celrep.2021.109549.

Giri B, Sharma P, Jain T, et al. Hsp70 modulates immune response in pancreatic cancer through dendritic cells. *Oncoimmunology*. 2021 Sep 18;10(1):1976952. doi:10.1080/2162402X.2021.1976952.

Ramakrishnan, S. HIF-2 in Cancer-Associated Fibroblasts Polarizes Macrophages and Creates an Immunosuppressive Tumor Microenvironment in Pancreatic Cancer. *Gastroenterology*. 2022 March 28, 162(7):1835-1837. doi:10.1053/j.gastro.2022.03.035.

Patents: None at the time of reporting.

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

Principal Investigator: Kunjan Dave, PhD

Organization: University of Miami

Summary: 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 staff 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. Research staff 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 last year of the project, the researchers were able to confirm that tumor necrosis factor alpha (TNF-α) inhibitor treatment prevented deleterious effects of chronic nicotine treatment in young animals of both sexes on TNF-α and blood brain barrier proteins level. Researchers also confirmed that chronic nicotine treatment lowers expression of blood brain barrier proteins in young female rats and chronic nicotine treatment impairs outcomes following autologous blood injection-induced intracerebral hemorrhage in young rats of both sexes.

Follow on Funding: Dave, KR,PI. Florida Department of Health James and Esther King Biomedical Research Program. 4/1/2024–3/31/2027. Submission date: 8/11/2023. Pending.

Dave, KR; PI. NIH/NINDS. R01 07/01/2023–06/30/2029. Submission Date: 10/05/2023. Pending.

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

Journals: Rehni AK, Cho S, Navarro Quero H, et al. Red Blood Cell Microparticles Limit Hemorrhage Following Intracerebral Hemorrhage in Spontaneously Hypertensive Rats. *Stroke*. 2023 Apr;54(4):e152-e154. doi:10.1161/STROKEAHA.122.042152.

Rehni AK, Cho S, Zhang Z, et al. Red Cell Microparticles Suppress Hematoma Growth Following Intracerebral Hemorrhage in Chronic Nicotine-Exposed Rats. *Int J Mol Sci*. 2022 Dec 2;23(23):15167. doi:10.3390/ijms232315167.

Patents: None at the time of reporting.

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

Principal Investigator: Nipun Merchant, MD

Organization: University of Miami

Summary: In this grant, the researchers show 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, researchers have shown that the stromal fibroblasts secrete IL6 as the major cytokine, which in turn, increases glycolysis in the pancreatic tumor cells, leading to increased lactate efflux in the microenvironment. The research team also shows that the secreted lactate because of metabolic reprogramming in response to stromal IL6 remodels the tumor immune microenvironment to make it immune evasive, by favoring M2 macrophages in the microenvironment. Furthermore, this Acidic pH and altered metabolic reprogramming cause production of oncometabolite named L-2Hydroxyglutarate (L-2HG) which drives stemness and aggressive phenotype in pancreatic cancer. The researchers show the treatment of pancreatic tumors with anti-IL6 antibody results in tumor regression as well as decreased CD133 + population within the tumor. Similarly, inhibiting the lactate efflux in the microenvironment decreases M2 macrophages and makes notoriously immune-evasive pancreatic tumors more responsive to anti-PD1 therapy. Overall, this study shows the importance of stromal cells in promoting tumor progression by enriching an aggressive and stem-like population within the tumor. It further shows that stroma can drive metabolic reprogramming and favor glycolysis in the tumor cells to promote increased efflux of lactate in the microenvironment, that can contribute to the immune-evasive property of the tumor. Finally, the study shows the researchers can target the IL6-induced lactate efflux from tumor to revert the immune-evasive microenvironment to an immune-supportive one and thus augment outcomes of checkpoint inhibitor therapy.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Kesh K, Mendez R, Mateo-Victoriano B, et al. Obesity enriches for tumor protective microbial metabolites and treatment refractory cells to confer therapy resistance in PDAC. *Gut Microbes*. 2022;14(1):2096328. doi:10.1080/19490976.2022.2096328.

Kesh K, Garrido VT, Dosch A, et al. Stroma secreted IL6 selects for "stem-like" population and alters pancreatic tumor microenvironment by reprogramming metabolic pathways. *Cell Death Dis*. 2020;11(11):967. Published 2020 Nov 11. doi:10.1038/s41419-020-03168-4.

Gupta VK, Sharma NS, Durden B, et al. Hypoxia-Driven Oncometabolite L-2HG Maintains Stemness-Differentiation Balance and Facilitates Immune Evasion in Pancreatic Cancer. *Cancer Res*. 2021;81(15):4001-4013. doi:10.1158/0008-5472.CAN-20-2562.

Patents: None at the time of reporting.

8. **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

Summary: Mammary specific polyomavirus middle T antigen overexpression mouse model (MMTV-PyMT) breast cancer tumor-bearing mice showcased cognitive deficits compared to non-tumor-bearing controls. Effective doses of 6.7mg/kg doxorubicin (DOX) and 66.7mg/kg cyclophosphamide (CYP) reduced tumor growth in this mouse model, yet persistent cognitive deficits arose from repeated administration. Both acute and repeated dosing of DOX and CYP led to elevated circulating Macrophage Inflammatory Protein 2 (MIP-2) (CXCL2) levels, an inflammatory marker known for its links to cognitive dysfunction and white matter damage. Furthermore, tumor-bearing mice inherently had augmented MIP-2 concentrations. Monocyte chemoattractant protein-1 (MCP1)'s Role: Tumor-bearing mice exhibited increased MCP1 (CCL2) levels, a protein connected to Alzheimer's disease progression and breast cancer lung metastasis. Remarkably, post-chemotherapy showed an even greater spike in MCP1, suggesting its potential role in chemotherapy-induced cognitive impairment. When mice exposed to chemotherapy were administered xanolamine or VU-357017, they didn't display the typical post-chemotherapy cognitive decline. However: Xanolamine prevented DOX+CYP-induced spatial memory impairment, but paradoxically induced memory deficits when given to otherwise unimpaired mice. This indicates that the benefit of xanolamine was not due to a direct effect of xanolamine improving cognition but rather by interfering with some adverse effect of DOX+CYP chemotherapy. VU-357017 did not demonstrate improvement in spatial memory post DOX+CYP and, in certain conditions, even appeared to induce cognitive deficits. Both Xanomeline and VU-357017 significantly decreased circulating Interleukin (IL-17) and granulocyte-colony-stimulating factor (G-CSF) levels. These compounds also diminished the pronounced G-CSF increase seen in tumor-bearing mice. Therefore, these drugs may have beneficial effects for the adverse effects of chemotherapy by interfering with neuroinflammation. Both compounds delayed the emergence of significant tumor growth and didn't interfere with the beneficial tumor-reducing impacts of DOX+CYP. This suggests that these drugs can be used as adjuvants that may improve the treatment of cancer as well as protect against cognitive deficits. This research has illuminated the nuanced cognitive impacts of chemotherapy within the MMTV-PyMT mouse model. Inflammatory markers linked to cognitive decline have been identified. While Xanolamine showed potential in mitigating chemotherapy-induced cognitive deficits, its adverse effects in unimpaired animals mandate careful consideration. Similarly, VU-357017's results were multifaceted, showcasing the intricate interplay between these compounds, chemotherapy, and cognitive health.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix Q: James and Esther King Biomedical Research
Fiscal Year 2022-2023 Closed Grants
Funded Fiscal Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
8JK03	H. Lee Moffitt Cancer Center and Research Institute, Inc.	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

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, Inc.

Summary: 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 pro-resolving 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 six months in former smokers, age ≥55 years, with lung nodules detected during LDCT screening program. Results of the proposed trial may have immediate and significant benefit to former smokers and other high-risk populations towards lung cancer prevention. In spite of the reduced funding, the goal is to obtain the safety and effectiveness of the combination of ω-3 FA + CUR or placebo in 100 men and women who are diagnosed with the lung nodules. 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

Summary: A major accomplishment during this project was the publication of the pre-clinical data supporting the rationale and importance of this proposed clinical phase one trial in a high impact clinical medicine journal. The research staff also developed a revised standard operating process (SOP) for efficiently expanding myxomavirus (MYXV) using the United States Food and Drug Administration (FDA) approved master cell bank A549 and HEK293T and using VERO cells for quantitative foci-forming units (FFU) assay. The research staff also submitted inquiries to the FDA (initially pre-Pre-Investigational New Drug Application (pre-IND)) and received notification to submit pre-IND inquiry with detailed information about the proposed clinical trial and strategy for collecting animal safety and biodistribution data for the IND application. Researchers then submitted a formal pre-IND application document and received favorable response from the FDA about the phase one clinical trial strategy along with detailed instructions regarding requirements for the IND submission. The main challenge for completion of this project was combined effect of the COVID pandemic followed by multiple personnel changes supervising the MYXV production within faculty of the University of Florida (UF) Powell Gene Therapy center. These challenges continued both during and after the COVID-19 pandemic that adversely impacted the main goal of the project which was generation of GLP (Good laboratory Practice) and GMP (Good Manufacturing) MYXV production. Specific challenges were detailed in quarterly reports.

Follow on Funding: None at the time of reporting.

Collaborations: This work includes collaborations between 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 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.

Appendix R: Live Like Bella Initiative
Fiscal Year 2022-2023 Newly Awarded Active Grants
Funded Fiscal Year 2022-2023

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
23L01	University of Florida	Jianping Huang, MD, PhD	$297,634.00	3/31/27	No	No	No
23L02	University of Florida	Jordan B. Milner, MD	$124,025.00	3/31/26	No	No	No
23L03	University of Florida	Brent Reynolds, PhD	$248,050.00	3/31/25	No	No	No
23L04	University of Florida	Steven Bruner	$248,029.00	3/31/25	No	No	No
23L05	University of Florida	Hugh Fan, PhD	$248,035.00	3/31/26	No	No	Yes
23L06	Florida State University	Jerome Irianto, PhD	$124,025.00	3/31/26	No	No	No
23L07	University of Central Florida	Annette Khaled, PhD	$248,050.00	3/31/25	No	No	No
23L08	Florida Atlantic University	Patrick Grant, PhD	$248,050.00	3/31/25	No	No	No
23L09	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Conor C. Lynch, PhD	$248,049.00	3/31/26	No	No	No
23L10	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Timothy Shaw, PhD	$122,714.00	3/3/26	No	No	Yes
23L11	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Damon Reed, MD	$297,629.00	3/31/27	No	No	No
23L12	University of Miami	Anis Ahmad, PhD	$248,050.00	3/31/26	No	No	No
23L13	University of Miami	Warren Alperstein, MD	$297,660.00	3/31/25	No	No	No

1. **Grant#:** 23L01 Phase I/II clinical trial for malignant pediatric gliomas using 8R-70CAR T cells

Principal Investigator: Jianping Huang, MD, PhD

Organization: University of Florida

Summary: Brain tumors are the leading cause of cancer-related deaths in children under the age of 20 in the United States. Among them, high-grade glioma (HGG) and a special category of gliomas, e.g., diffuse intrinsic pontine gliomas (DIPGs), are devastating; their five-year survival is less than 20%. Cancer immunotherapy is one of the most promising new treatment strategies for these patients. However, very few tumor-specific targets have been discovered in brain tumors. Researchers have developed a new technique, using patients' own cells in the blood, called T cells, to make a tumor-killing drug. Research staff first found a specific tumor target, which is expressed on the surface of the brain tumor cells, called CD70. The research team engineered the patients' T cells in the laboratory with a particular receptor called a chimeric antigen receptor (CAR) and let them attack the CD70-positive cancer cells. To help these T cells to find the tumors, researchers installed a 'GPS' in the T cells to guide them to the tumor zone, (the drug is called 8R-70CAR T cells). The results show that engineered T cells can cure 100% of mice with glioblastoma in their brains and protect them from tumor regrowth. The significance of this research is that it may not only advance a new therapy for brain tumors in children but also provide a strategy of treatment applicable to many other CD70-positive pediatric cancers, including leukemias, lymphomas, Ewing' sarcoma, osteosarcomas, etc. The 8R-70CAR T cell platform was approved by the FDA in a phase I trial for adult patients with newly diagnosed GBM (NCT05353530). After treating 3-6 adult patients in the phase I adult

trial, this phase I/II trial for pediatric HGG/DIPG will be initiated, and up to 36 patients will be enrolled. Researchers anticipate starting it in mid-2023.

Collaboration: The University of Florida (UF) Brain Tumor Immunotherapy Program maintains a full-time staff of clinical trials coordinators with considerable experience in clinical trials management. A designated coordinator supported by the Brain Tumor Center is assigned to each clinical protocol involving the enrollment of human subjects. A team member in the University of Florida Health Cancer Center (UFHCC) provided support for comprehensive patient care and innovative research in a collaborative, multidisciplinary environment. The UFHCC has a membership of more than 280 researchers and clinicians across the University of Florida and UF Health, the Southeast's most comprehensive academic health center. The UFHCC and its members provide leading-edge cancer care and conduct original research for cancer prevention, early diagnosis, and treatment. The ReMission Alliance has attracted the attention of top brain cancer experts from across the globe, creating a network of neuro-oncology, tumor immunology, and genetics experts. This group has been vested by top peer institutions and a community of vested collaborators and influencers affected by brain cancer. The Alliance headquarters runs out of the University of Florida Health in Gainesville. A potential collaboration with Pacific Pediatric Neuro-Oncology Consortium (PNOC) will be accomplished. These networks will help ensure this pediatric trial is initiated and conducted successfully.

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#:** 23L02 Utilization of Alpha/Beta T Cell and B Cell Depletion in Allogeneic Stem Cell Transplantation in Malignant Diseases

Principal Investigator: Jordan B. Milner, MD

Organization: University of Florida

Summary: Allogeneic stem cell transplantation (AlloSCT) is a curative treatment for malignant and non-malignant diseases. A HLA-matched sibling (MSD) is an optimal AlloSCT donor for patients with malignant and non-malignant diseases; however 75% of AlloSCT recipients require an alternative donor source, as they do not have a MSD available. AlloSCT is limited by graft versus host disease (GVHD) which is a major cause of non-relapse mortality. Recent review from the Florida Pediatric BMT and Cell Therapy consortium indicated 12% incidence of clinically significant GVHD in children with NMD receiving alloHCT in Florida. A review of adult CIBMTR data (1999 to 2005) revealed a cumulative incidence of a GVHD grade II-IV to be 38% in MSD transplantations at 100 days and can affect 40% to 60% of alloSCT recipients while contributing to 15% of deaths. Chronic GVHD (cGVHD) is a life-threatening complication and approximately 50% of alloSCT patients will develop cGVHD despite their donor source. A recently developed technique uses α/β CD3+ T-cell and CD19+ B-cell depletion from peripheral blood stem cells to permit rapid platelet and neutrophil engraftment with decreased risk of GVHD, and more rapid immune reconstitution. B-cells are depleted in order to prevent post-transplant lymphoproliferative disorders (PTLD) and decrease risk of cGVHD. This technique

uses the Miltenyi CliniMACS Prodigy® system using magnetic beads to negatively select α/β+ T-cells and CD 19+ B-cells, permitting other cell types to remain in the product. The research team hypothesizes that pediatric alloSCT recipients utilizing α/β CD3+ T-cell and CD19+ B-cell depletion for malignant diseases will have a decrease in the incidence of GVHD while maintaining a comparable overall survival compared to MSD. This will be a single institution, prospective, phase 2 trial. Patients less than 30 years of age with a malignant disease who meet eligibility can undergo stem cell transplantation utilizing α/β CD3+ T-cell and CD19+ B-cell depletion. Eligible diseases include acute leukemia, juvenile myelomonocytic leukemia, myelodysplastic syndrome, lymphoma, or high-risk solid tumors. Patients can receive an HLA-matched (5-6/6) sibling donor, matched (8-10/10) unrelated donor available for stem cell donation, or haplo-identical familial donor (³3/6 HLA match). Patients will receive conditioning as appropriate for their disease, disease status, and organ function. All products will undergo α/β CD3+ T-cell and CD19+ B-cell depleted using the investigational CliniMACS Prodigy® according to the manufacturer's instructions. Products will have strict release criteria prior to being approved for patient infusion. Patients will receive a minimum cell dose of 5 x 106 CD34+ cells/kg with a maximum of 20 x 106 CD34+ cells/kg. All patients will be monitored for myeloid and platelet engraftment, development of GVHD, engraftment failure, infections, and development of PTLD using Kaplan-Meier plots. Pediatric patients with malignant diseases who receive alloSCT will have access to advanced care affording them the opportunity to have an improved quality of life with a low risk of GVHD, engraftment failure, delayed immune reconstitution, no increased risk of infection or PTLD, and an improved graft versus leukemia effect, as compared to a non-manipulated graft.

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#:** 23L03 Intranasal delivery of BMP-4 using plant-derived extracellular vesicles for treatment of pediatric glioma

Principal Investigator: Brent Reynolds, PhD

Organization: University of Florida

Summary: The current project aims to utilize a unique method for transporting a protein to tumor cells, employing plant-derived extracellular vesicles. The function of this protein is not to destroy the tumor cells but to transform them into non-harmful entities. To address brain tumors specifically, the protein uses a nose-to-brain route, thereby circumventing the blood-brain barrier that often impedes therapies. Success in this endeavor could signify substantial progress in treating pediatric brain cancers. Six varieties of hemp plants serve as sources for this research, from which extracellular vesicles have been successfully isolated. Nanotracking analysis has allowed for a detailed examination of the size and concentration of these vesicles. Additionally, the inclusion of a part-time researcher contributes to the daily research operations. Collaboration with multiple research groups facilitates further examination of the hemp vesicles. The upcoming stages in this project will involve a comprehensive analysis of these vesicles, investigating their cannabinoid content, protein components, and RNA analysis. No unexpected

delays or obstacles have occurred, suggesting the planned objectives are achievable. Childhood brain tumors, despite being the second most prevalent type of cancer among children, unfortunately, lead to the highest number of cancer-related fatalities. The existence of the blood-brain barrier, a selective barrier that hampers many drug deliveries to the brain, amplifies the treatment challenge. Additionally, the young age of patients often results in severe treatment side effects. Consequently, an urgent need exists for safe, specialized treatments for pediatric brain cancers. This research may offer innovative, less harmful treatments for childhood brain cancers, potentially enhancing survival rates and quality of life for Florida's pediatric population.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

4. **Grant#:** 23L04 Functional role of the srp54 gene in pediatric leukemia and bone marrow failure syndromes.

Principal Investigator: Steven Bruner

Organization: University of Florida

Summary: Inherited pediatric bone marrow failure syndromes are disorders characterized by altered production of specific bone marrow cell linages and are cancer predisposition syndromes. These disorders are commonly associated with pediatric myelodysplastic syndrome and leukemia. Blood cancers can be a direct result of malignancies or as a consequence of therapeutic treatment. Specific mutations in the 54-kDa signal recognition particle (SRP54) gene were recently shown to result in severe congenital neutropenia, as well as a Shwachman-Diamond like syndrome. SRP54 mutations have been demonstrated to cause neutropenia and aberrant splicing of X-box binding protein 1 in zebrafish and human cells. Patients with SRP54 deficiency often fail to respond to the primary treatment, granulocyte-colony stimulating factor (G-CSF), to improve their neutrophil counts, have a high incidence of recurrent infection and have an increased chance of developing leukemia. SRP54 is an essential component of the co-translational complex that targets secretory and membrane proteins to the endoplasmic reticulum. This project will use the research group expertise in synthetic biology and enzyme structure/mechanism to study SRP54 function/dysfunction. This program is a new research direction for the group, and the award will initiate a long-term program focused how SRP54 mutations affect neutrophil progenitor cells and lead to leukemia. The specific aim of the project is to develop novel assays to study the function of SRP54, along with detrimental mutations. The methodology will focus on the evolution and selection of SRP54 mutants with altered phenotypes. Specific congenital SRP54 mutations are known, but how these mutations affect SRP54 function, along with the ability to predict leukemia predisposition, are not established. The research group's experimental results can be compared to clinical sequencing data to provide insights into the genetic basis for the disorders and leukemogenesis. Yeast will be used as a model system and the primary aim is the discovery of rescue phenotypes of clinically relevant SRP54 mutations. The groups will establish a continuous evolution approach and link mutations that rescue SRP54 function to a fluorescent signal that can be quantified on the yeast

cell surface. This high-throughput experiment will provide sequence-function relationships, enabling the groups to map the fitness landscape of SRP54. Overall, this proposal will establish a high throughput assay to probe the role of SRP54 in the secretory pathway and pediatric cancers. The assay will be used to both examine SRP54 function/disfunction and screen small molecule libraries toward the discovery of molecular probes and therapeutic lead compounds.

Follow on Funding: None at the time of reporting.

Collaborations: The groups have initiated a collaboration with Prof. Akiko Shimamura, director of Dana-Farber/ Boston Children's Cancer and Blood Disorders Center.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

5. **Grant#:** 23L05 Towards liquid biopsy for pediatric sarcoma

Principal Investigator: Hugh Fan, PhD

Organization: University of Florida

Summary: The overall goal of this project is to study circulating tumor cells (CTCs) as a biomarker of liquid biopsy, which is a laboratory assay that analyzes tumor-derived materials in blood of cancer patients. Liquid biopsy is considered an alternative to tissue biopsy that is the current standard for cancer diagnosis and prognosis. Advantages of liquid biopsy include being minimally invasive, easy sample access through a routine phlebotomy, and more frequent assay than tissue biopsy for monitoring the treatment response. Since sarcoma are targeted in this project, the primary population who will benefit from this research is sarcoma patients. However, about 20% of pediatric cancer are sarcomas. Note that this research can be adapted for other types of cancer, thus the general population could benefit in the future if liquid biopsy can serve as a non-invasive cancer screening tool. The progress during this report period includes studying surface proteins of CTCs. The current CTC definition approved by U.S. Food and Drug Administration (FDA) relies on cytokeratins (CKs) 8, 18, 19. Research staff compared CK (targeting CKs 7 and 8) with panCK (targeting a wider range of CKs). Samples were collected from CK-positive sarcoma subtypes, including synovial sarcoma (SS) and desmoplastic small round cell tumor (DSRCT). Each sample underwent parallel processing using two microfluidic devices. After CTC isolation, the microfluidic devices were subjected to immunofluorescence staining for CTC detection, with each device using a different protein marker: CK or panCK, in addition to other protein markers defined by the United States Food and Drug Administration (FDA). The research staff found that each sample has higher CTCs using panCK than using CK, indicating that the expansion of protein marker coverage significantly affects CTC detection in CK-positive sarcoma. Overall, research staff have made nice progress towards the goals of this project.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Le, M. N., Smith, K. A., Alipanahrostami, M., Chen, K., Lagmay, J. P., Fan, Z. H., Microfluidics-Enabled Isolation and Single-Cell Analysis of Circulating Tumor Cells, *Methods in Molecular Biology*, 2023, 2689: 71–93. Doi:10.1007/978-1-0716-3323-6_7.

Patents: None at the time of reporting.

6. **Grant#:** 23L06 Evaluation of LARP6 inhibitor for the treatment of pediatric glioblastoma

Principal Investigator: Jerome Irianto, PhD

Organization: Florida State University

Summary: Brain tumors are the second most common form of cancer in children. Glioblastoma, one of the most aggressive types of brain tumors, occurs in 3-15% of pediatric patients and the five-year survival rate is less than 20%. New therapeutic approaches are urgently needed to improve the poor prognosis of the disease. A recent study on glioblastoma showed the critical role of type I collagen produced by the tumor cells, in promoting disease progression and invasive capabilities. The level of collagen type I alpha 1 chain (COL1A1) expression also correlates with the poor prognosis of the patients. These results suggest that inhibition of collagen I synthesis in the glioblastoma cells might be a potential therapeutic approach to inhibit glioblastoma progression. Researchers have recently shown that la ribonucleoproteins (LARP6) regulates the translation of collagen I messenger ribonucleic acid (mRNAs) by directly binding to these mRNAs. In fact, treatment with LARP6 inhibitor at low doses suppressed collagen synthesis and hepatic fibrosis, in animal models. The biosynthesis of type I collagen is highly conserved in all collagen-producing cells, hence researchers expect that LARP6 inhibition will suppress type I collagen production by glioblastoma cells. Indeed, COL1A1 and LARP6 are expressed in pediatric glioblastoma, and the preliminary data has shown that inhibition of LARP6 binding suppresses the growth of pediatric glioblastoma organoids, three dimensional (3D) miniature versions of glioma produced in vitro. This growth suppression was achieved using nanomolar concentrations of a recently discovered LARP6 binding inhibitor, which makes it a promising lead compound for first-inclass anti-glioma drugs. Based on these findings, researchers hypothesize that LARP6 inhibition suppresses pediatric glioblastoma progression and its potential to metastasize. The reseach team will assess the dose-dependent effect of the LARP6 inhibitor on pediatric glioblastoma organoid growth rate, cell migration potential, expression of COL1A1, and epithelial to mesenchymal transition markers. The research team will elucidate the mechanism of action of LARP6 inhibitor in pediatric glioblastoma progression by analyzing the mechanistic changes in type I collagen biosynthesis within pediatric glioblastoma organoids and correlate them to organoid growth. Additionally, gene expression changes will be profiled to assess the global impact of LARP6 inhibition on the characteristics of pediatric glioblastoma organoids. Combining the available expertise in cancer cell biology and LARP6 biology, researchers will verify the impact of LARP6 inhibition on the progression of pediatric glioblastoma. In completing this research, the research team will reveal the potential of the LARP6 inhibitor as a novel anticancer drug for the treatment of pediatric glioblastoma. Importantly, this project is highly translational; that verifying the LARP6 inhibitor compound as a promising anticancer drug will advance it into structure-activity relationship analysis and preclinical studies. In addition, this research on pediatric glioblastoma will contribute to the broader field of cancer therapy by exploring the use of LARP6 inhibitors for cancer 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.

7. **Grant#:** 23L07 Targeting Chaperonin-containing TCP-1 for the Treatment of Neuroblastoma

Principal Investigator: Annette Khaled, PhD

Organization: University of Central Florida

Summary: Neuroblastoma (NB) is a spectrum of neuroblastic cancers that arise from the abnormal growth of embryonic neural crest cells and is the most common cancer diagnosed in children under one year of age. The five-year survival for children with disseminated high-risk NB is 40-50%. These patients may suffer from severe toxicities due to aggressive multimodality therapies that lead to long-term morbidity and mortality concern. The molecular factors responsible for NB are poorly understood and few druggable targets are known. To address this unmet medical need, the research objective is to therapeutically inhibit NB by targeting the process of protein folding by molecular chaperones. Chaperones enable the cancer phenotype to emerge from the mutated genotype and are ideal targets for therapeutic intervention since cancer cells become highly reliant on chaperones, to the point of addiction. The challenge is to inhibit chaperones in NB cells without harming healthy cells. To that end, the research staff discovered that a protein folding complex, called Chaperonin-Containing TCP-1 (CCT), is highly expressed in NB, correlates with invasiveness, and inversely correlates with patient survival. In healthy cells, CCT may fold only 1% of the cellular proteome. In cancer cells, CCT folds proteins involved in the signal transduction pathways that promote cancer. CCT interactors are found in the ten major signaling pathways known to cause cancer (e.g., cell cycle, p53, MYC, AKT, RAS, Hippo, etc.). researchers hypothesize that NB cells upregulate the protein folding activity of CCT to meet the needs for specific protein substrates that enable NB progression and metastasis. This innovative hypothesis is supported by the data showing that the second CCT subunit (CCT2) is essential for the survival and invasiveness of NB cells by increasing the protein folding activity of CCT to generate substrates like actin and tubulin to support migration, a biomarker for the detection of circulating NB cells in blood, and amenable to inhibition using CT20p, a CCT inhibitor developed by the lab, initially tested using nanoparticles (NPs) as delivery vehicles .To efficiently deliver CT20p to NB, researchers will use bioengineered intracellular bacteria instead of NPs. Such bacteria are not limited by challenges associated with NPs, such as degradation during circulation, poor uptake into cancers, or failure to escape endosomes, and are attracted to the compounds produced by cancer cells, efficiently delivering macromolecules, like CT20p, to tumors. In this project, research staff will bioengineer a novel Salmonella strain that secretes CT20p in an inducible and self-limiting manner to impair CCT protein-folding activity and cause NB cell death, that will be safe to use, and will produce few off-targets effects by concentrating CT20p in NB tumors. An IL-2 secreting form of Salmonella (Saltikva) was deemed safe in human phase I clinical trials of metastatic solid tumors, supporting clinical translation of the research. Outcomes will be therapeutic bacteria that produce few adverse effects in patients and have promising use for oral or systemic delivery of CT20p in the treatment of NB.

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#:** 23L08 Identification of epigenetic mechanisms of resistance to chemotherapy in pediatric acute lymphoblastic leukemia

Principal Investigator: Patrick Grant, PhD

Organization: Florida Atlantic University

Summary: Pediatric acute lymphoblastic leukemia (ALL) is the most common cancer diagnosed in children. It is a type of cancer in which the bone marrow makes a large number of abnormal blood cells. The overall survival rates of children with ALL have improved over recent years, driven by chemotherapy intensification, stem cell transplantation, and improved supportive care. The current five-year survival rate range is around 90%. The main treatment strategy involves phases of chemotherapy, including courses that may include cytarabine in high-risk patients. Despite advancements in chemotherapy and supportive care, primary refractory or relapsed pediatric ALL has resulted in significant morbidity and mortality. A wide range of outcomes exist for the different subtypes of ALL and very high-risk patients continue to have poor outcomes with event free survival rates below 50%. As a result it remains critical to identify new targets of therapy, particularly in high-risk patients. It has been proposed that non-genetic or so-called epigenetic abnormalities have an important role in the plasticity of cell states during cancer progression, and that this could lead to drug resistance. Therefore, researchers posed the question as to whether there is a potential drug-targetable epigenetic regulator whose inactivation would make chemotherapy more effective? In order to identify such mechanisms of resistance, researchers used a novel approach to investigate multiple epigenetic pathways of resistance to cancer drugs in a model system. Research staff have identified one novel pathway that is critical for cellular resistance to platinum with cross-resistance to other chemotherapeutic agents. In a limited study of ALL samples researchers discovered that a candidate driver of this pathway leads to a far worse outcome when elevated in pediatric patients, and a reduction of median overall survival of around 30 months. The research team believes that one histone modification in particular potentially plays a pivotal role in the resistance to cancer treatment. The research staff propose to further characterize this target of drug resistance in the context of pediatric ALL. In addition, using the same model system researchers propose to screen for the interruption of multiple epigenetic signals for enhanced sensitivity to other drug treatments such as cytarabine, also known as Ara-C. Collectively this research aims to identify new drug targets for the treatment of pediatric ALL, which also has possible ramifications for adult leukemias and other cancers.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

9. **Grant#:** 23L09 Identifying How HDAC Suppression of SLC17A7 Drives Osteosarcoma Progression and Metastasis

Principal Investigator: Conor C. Lynch, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: Osteosarcoma is a rare but deadly disease that affects mostly children and adolescents. The majority of osteosarcoma patients die because of metastatic disease. Metastasis is the process whereby cancer spreads from the primary site (the bone) to a secondary organ. The lungs are the most common site of osteosarcoma metastasis. Treatment consists of surgery and/or chemotherapy with methotrexate, cisplatin, and doxorubicin mix. While treatment options for patients with other types of cancers has grown, osteosarcoma has, frustratingly, been left behind. This is in part due to the rarity of the disease but also to the fact that there is little pre-clinical experimental data generated with therapies that can convince medical oncologists to conduct human trials. The objective of this research project is to meet the urgent need for pre-clinical data to motivate human clinical trials. To this end, researchers have tested a number of drugs for their ability to kill osteosarcoma cells. Importantly, these drugs are approved by the Food and Drug Administration (FDA) for the treatment of other cancers but not for osteosarcoma. The preliminary data shows that osteosarcoma is very sensitive to histone deacetylase (HDAC) inhibitors. HDACs are enzymes that switch on or off the expression of many genes that can promote or prevent cancer growth respectively. On their own, HDAC inhibitors can also have toxicities for patients but the data show that very low doses of HDAC inhibitors can work effectively with low doses of chemotherapies such as methotrexate. Researchers expect lower dose regimens would mean lower toxicities for patients. Also, to identify additional drug targets, the research team asked what genes are controlled by HDACs. The data show that HDACs may mediate their effects by switching off solute carrier family 17 member 7 (SLC17A7). SLC17A7 transports an amino acid known as glutamate into tiny vessels within the osteosarcoma cell. Researchers suspect that trapping glutamate in these vessels robs osteosarcoma of an important building block and promotes its death. Researchers aim to test if this is the case. Excitingly, a therapy known as Riluzole that blocks glutamate use exists and is FDA approved (for the treatment of Lou Gehrig's disease). The data provide rationale for the hypothesis that: HDACs contribute to osteosarcoma progression and metastasis via suppression of the glutamate transporter SLC17A7. Researchers will test this hypothesis with three aims. Researchers will determine how does SLC17A7 control osteosarcoma cell growth. Researchers will then interrogate if SLC17A7 is necessary for osteosarcoma progression and metastasis in mice, while research staff will also examine if the administration of low-dose HDAC inhibitors improve the efficacy of single agent chemotherapy. Scientifically, researchers expect that the results will greatly advance the knowledge of how SLC17A7 contributes to osteosarcoma biology via regulating glutamate availability. Translationally, it is expected that these results will motivate clinical trials for children and young adults with lung metastatic osteosarcoma in the short term since the therapies being focused on are FDA approved.

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#:** 23L10 Targeting ER Stress in Pediatric Acute Myeloid Leukemia

Principal Investigator: Timothy Shaw, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: There is a critical need to identify novel therapeutic strategies, especially in acute myeloid leukemia (AML), which remains the most refractory leukemia in children with minimal progress in therapy or outcome in over 30 years. This is especially true in leukemia patients bearing Mixed Lineage Leukemia 1 (MLL)-rearranged gene fusion, which is associated with a poor prognosis, thus underscoring the urgent need for novel therapies targeting specific fusion subgroups. The unfolded protein response (UPR) through the mediator protein kinase ribonucleic acid (RNA)-like endoplasmic reticulum kinase (PERK) has been proposed as a mechanism for drug resistance by regulating translation, autophagy, and cellular survival. Through preliminary analysis of the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) 1031 AML cohort, researchers found that a high expression of the PERK stress signature (250 PERK-related transcripts) is associated with high-risk MLL-rearranged patients. With clinical interest emerging in applying PERK inhibitors (PERKi) in adult tumors, researchers hypothesize that PERK inhibition may be applicable as a novel approach to overcome resistance in refractory AML patients. The research team will examine the effect of PERK dysregulation in MLL-rearranged AML cells and hypothesize that AML cells enhance viability through PERK in the presence of ER stressors and anti-AML inhibitors. The research team will generate clustered regularly interspaced short palindromic repeats (CRISPR) knockout models of AML cells as well as cell lines treated with PERK short hairpin ribonucleic acid (shRNA) followed by endoplasmic reticulum (ER) stressor or anti-AML inhibitors (such as HDAC inhibition). The research team will examine cell death by flow cytometry and proliferation by Cell Titer Glo and will then characterize the PERK-dependent translatome in drug-treated AML cells. Researchers hypothesize that AML cells enhance drug resistance through the PERK-dependent translatome and will treat AML cell lines with PERK inhibition (Amgen's molecule (AMG44), a highly specific PERK inhibitor; and GSK-2606414, a dual PERK and RIPK1 kinase inhibitor). Researchers will then perform bulk proteomics profiling, polysome and ribosome-sequencing. The research team will use signatures based on the three major arms of the ER stress pathways: inositol requiring enzyme-1 (IRE1), PERK, and activating transcription factor 6 (ATF6), train a Deep Learning model and apply it in a retrospective cohort from the National Cancer Institute (NCI) TARGET and patients from the H. Lee Moffitt Cancer Center patient network. Special attention will be placed on a novel B-cell CLL/lymphoma 11b (BCL11B) leukemia subgroup, which is presented with a mixed-lineage phenotype with an unknown oncogenic mechanism. The emerging evidence for PERK-related UPR activation as a key driver of resistance is a novel and exciting area with tremendous potential for therapeutic intervention in AML. This study will provide a valuable resource for studying ER stress in AML while revealing novel insights that leads to the development of new approaches to overcome resistance in AML as well as in other pediatric malignancies.

Follow on Funding: None at the time of reporting.

Collaborations: Darwin Chang is receiving training in computational biology and performing the bench experiments under the research project.

Journals: Obermayer AN, Chang D, Nobles G, et. al. PATH-SURVEYOR: pathway level survival enquiry for immuno-oncology and drug repurposing. *BMC Bioinformatics*. 2023 June 28;24(1):266. doi:10.1186/s12859-023-05393-y.

Patents: None at the time of reporting.

11. **Grant#:** 23L11 Feasibility of Generating Novel Translational and Therapeutic Strategies based on a Multicenter, Pediatric and AYA Evolutionary Tumor Board; pedsETB

Principal Investigator: Damon Reed, MD

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: Cancer is complex. There are often a mix of cells with varying degrees of growth potential, ability to spread (metastasize) and to resist chemotherapy. The combination of these factors leads to the eventual outcome. What has stood out to this group of investigators for the past few years has been that while everyone can agree cancer is complex, researchers have not figured out how to make this therapeutic approach match this complexity. Typically treatment starts with shrinking the tumor, but will continue that when the tumor is gone, even when it is suspected that the unseen tumor will most likely come back. This team posits that the typical therapy should change because the cancer is changing. No cancers perhaps better fit the bill for a need for daring and complex decision making to improve outcomes than pediatric solid tumors. These cancers have fallen way behind in terms of cure rates over the past three decades of cancer discovery. Efforts at Moffitt Cancer Center to model cancer as adaptive populations of heterogeneous cancer cells populations have yielded some surprising and hopeful directions to better anticipate and think ahead when treating these complex cancers that respond but then fail when treated with a repetitive therapy. The ongoing, adult Evolutionary Tumor Board (ETB) has met monthly for the past two years and has high demand. ETB allows researchers to prospectively expand clinical decision making with members of a team including clinical oncologists, mathematicians, and evolutionary biologists. The research staff are investigating if novel treatment strategies can be generated by presenting patients across the cancer center who lack curable strategies with the majority of enrolled patients having solid tumors such as sarcomas and head and neck cancers. The treating physician provides a real patient scenario after obtaining consent, and the trajectory of cancer with and without past treatments is modelled. The ETB makes a recommendation and tracks if the ideas are accepted by the physician, presented as an option to a patient, and implemented or not. ETB recommendations are not binding and the clinical care is delivered at the discretion of the treating physician. ETB aims to provide an idea that can serve as an additional option for providers or patients. The ideas have stimulated preclinical experiments and have led to the development of multiple clinical trials to investigate an idea further and more formally. Demand for ETB has been great including outside Moffitt Cancer Center. Rather than wait for ETB to expand in adults, the researchers are aiming to expand to Florida centers and Duke, St. Jude, Nationwide Children's and Cleveland Clinic to bring pediatric ETB (pedsETB) as an available resource for patients lacking curative strategies.

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#:** 23L12 Preventing treatment-induced nephrotoxicity in pediatrics cancer survivors

Principal Investigator: Anis Ahmad, PhD

Organization: University of Miami

Summary: Cytostatics, abdominal radiotherapy, total body irradiation (TBI), and some agents used in supportive care may induce acute kidney injury (AKI) or lead to chronic kidney disease (CKD) in pediatric cancer survivors. As the clinical management of pediatric cancer improves and more patients survive, there is a critical need to assess the quality of life of long-term pediatric cancer survivors and reduce treatment-induced normal tissue toxicities. For most pediatric acute myeloblastic leukaemia (AML) HSCT indications, chemo conditioning gained preference over TBI-based conditioning. Studies consistently showed superior survival outcomes of TBI-based conditioning in children with very high-risk acute lymphoblastic leukemia (ALL). Similarly, platinum agents may also cause dose-dependent acute and chronic glomerular and tubular damage, affecting more than 60% of children and as many as 90% of adults five years after treatment. Very young children (below three to four years) are more prone to developing severe side effects from HSCT and TBI-based conditioning. TBI-based conditioning causes more late effects than chemoconditioning.Treatment-induced nephrotoxicity is an irreversible serious late complication that produces renal impairment, negatively impacting pediatric cancer survivors' quality of life. No effective clinical treatments exist to prevent treatment-induced nephropathy. The research team recently discovered and characterized ATP Binding Cassette Subfamily A Member 1 (ABCA1), a gene that exports cholesterol from podocytes. The preliminary data demonstrate that after radiation therapy, ABCA1 expression decreases in a dose- and time-dependent manner in cultured podocytes, which coincides with decreased cholesterol efflux and increased podocyte apoptosis. In contrast, overexpression of ABCA1 in vitro protects from radiation-induced podocyte apoptosis. Research staff also found that focal bilateral kidney irradiation of C57BL/6 mice with or without cisplatin induces lipid accumulation, Nlrp3, IL-1β, IL-18r1, Nfkb1, Tlr4, Casp4 messenger ribonucleic acid (mRNA) expression, and GBM thickness that correlated with decreased ABCA1 expression, podocyte number, and glomerular filtration rate. Based on the preliminary findings, researchers hypothesize that intracellular cholesterol accumulation due to ABCA1 deficiency promotes treatment-related chronic kidney disease progression, and pharmacological induction of ABCA1 or cholesterol depletion with cyclodextrin can prevent treatment-related nephrotoxicity.The objective is to investigate the mechanistic role of ABCA1 in renal injury after single-dose TBI and fractionated TBI conditioning with or without chemotherapy before hematopoietic stem cell transplantation (HSCT), as this represents a standard of care for conditioning. The long-term goal is to discover the mechanism of renal damage after HSCT transplantation and improve the quality of life of leukemia and lymphoma pediatric cancer survivors.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

13. **Grant#:** 23L13 Investigating the impact of demographic factors on the development of GVHD

Principal Investigator: Warren Alperstein, MD

Organization: University of Miami

Summary: Hematopoietic stem cell transplant (HSCT) continues to grow as a curative option for many pediatric malignancies and some other rare pediatric diseases such as sickle cell anemia. For more than 60 years, HSCT application has been limited by graft versus host disease (GvHD), a severe complication frequently associated with high morbidity and mortality. GVHD develops in approximately 40% to 60% of transplant recipients despite standard prophylaxis. Although the likelihood of GVHD is directly related to the degree of compatibility between patients and donors, patients receiving HLA-identical grafts are also at risk for developing GVHD symptoms. While a few clinical factors, such as age, pretransplant condition and degree of HLA match, have been identified as predictors of GVHD, none of them have been reliable in clinical practice. Unfortunately, once symptoms occur GvHD is not easily treated. First line therapy consists of corticosteroids, however, a significant number of patients present as steroid-resistant or steroid-dependent. No consensus exists for second line therapy and management strategies differ significantly between treatment centers and physicians. Similar to GVHD development, no predictors for steroid response have been validated to date.This proposal aims to investigate demographic and treatment-related predictors of GVHD development and steroid treatment response. South Florida represents a unique and diverse demographic catchment area and this diversity allows research staff to investigate GVHD in patients of different demographic backgrounds that have been treated with a variety treatment modalities throughout their cancer and GVHD journey. Researchers will evaluate a set of historic patient data in order to identify factors that may predict for the development of GVHD and resistance to GVHD treatment.Additionally, the research team will prospectively collect blood samples from a small set of transplant patients at different time throughout their treatment journey to evaluate steroid sensitivity prior to GVHD onset and during GVHD treatment. This approach will allow staff to prospectively monitor changes in steroid treatment response through changes in activated T-cell viability in response to treatment. T-cell response to steroid treatment will be evaluated a previously established and validated drug sensitivity testing platform. The ability to use a precision medicine approach to predict steroid treatment response on a per patient basis would represent a major advancement and open the door for future use of this platform for GVHD drug development and patient stratification.The researchers believe this is an extremely innovative approach, which if effective could greatly impact patient care.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix S: Live Like Bella Initiative
Fiscal Year 2022-2023 Active 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	Yes	No	Yes
22L04	University of Central Florida	Alicja Copik, PhD	$250,000.00	3/31/25	No	No	Yes
22L05	University of Central Florida	Griffith Parks, PhD	$250,000.00	3/31/25	No	No	Yes
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	No	No

1. **Grant#:** 22L01 Personalizing radiotherapy dose using genomic markers of radiosensitivity to predict tumor response and normal tissue toxicity an pediatric malignancies.

Principal Investigator: Matthew Hall. MD

Organization: Baptist Health South Florida

Summary: The purpose of this project is to perform a first in children study of radiosensitivity index (RSI) and genomic-adjusted radiation dose (GARD), which are prognostic and predictive biomarkers of local tumor control and overall survival in multiple adult cancers. These metrics have not been previously studied in children, many of whom use radiotherapy as part of curative intent treatment. Targeted therapies are increasingly used to personalize cancer care based on the individual patient's tumor genome. While radiotherapy technologies have advanced over time, patients largely receive the same empirical radiation dose based on diagnosis and risk of normal tissue complications. In adults, the study team at Moffitt developed RSI to stratify radiosensitive versus radioresistant tumors and GARD to quantify the optimal RT dose for individual patients to achieve local disease control. These measures are calculated using results from a multigene expression panel to predict tumor radiosensitivity. RSI and GARD were previously validated in adults but have never been studied in pediatric cancers. This grant was awarded in February 2022 and the terms and conditions were signed on March 11, 2022. Institutional review board (IRB) approval was obtained on July 13, 2022. The study was activated at Miami in August 2022. At this report, approximately 135 evaluable patients have enrolled out of the planned sample size of 200 patients. Of note, enrollment has accelerated during 2023, which is attributed to augmenting patient recruitment using electronic consent documents and leveraging the pediatric oncology and multidisciplinary survivorship clinics to identify and enroll patients in addition to the radiation oncology clinic. Electronic data capture and collection of pathology specimens has proceeded for enrolled patients. As of this report, the first cohort of patient pathology specimens was shipped to the research lab at Moffitt for gene assay testing, which is needed to calculate RSI and GARD. If validated in children, RSI and

GARD may enable pediatric cancer patients to receive personalized radiation doses that are individualized based on their tumor genome. In childhood cancer patients, this may help to reliably achieve cures while reducing the risk of normal tissue injury from radiotherapy. Childhood cancer survivors experience significant health complications that may be fatal after treatment; reducing these effects is imperative to help survivors succeed in school and live independently in the future. In tumors that are radiosensitive, some patients may safely receive lower radiation doses, maintain high cure rates, and reduce the risk of late toxicities. In radioresistant tumors, physicians may be able to dose escalate in molecularly high-risk patients to improve local control and survival. This may enable pediatric radiation oncologists to intelligently personalize radiotherapy in children for the first time.

Follow on Funding: None at the time of reporting.

Collaborations: This research project is a combined effort of Miami Cancer Institute and Moffitt Cancer Center. The research study team has greatly benefited from the work of Keitrina Mair, a premedical student from Barry University in Hollywood, Florida who is completing a one-year research program in the department of radiation oncology at Miami Cancer Institute in September 2023. Specifically, Keitrina has been instrumental in screening the long-term follow up clinic lists in radiation oncology, survivorship, and pediatric oncology to identify potential patients who are eligible to participate in this study. Keitrina is primarily responsible for electronic data capture for patients who have enrolled on the trial and has entered data for the Biospecimen Repository team on the location and details of the pathology specimens that must be collected for this project. During the last three months, Keitrina has been responsible for attending the pediatric oncology clinic on a weekly basis to identify potential patients and has assisted the clinical team in obtaining consents in this clinic. Finally, Keitrina has helped to identify patients and families who have agreed to participate and been sent electronic consent forms to be completed but have not returned their permissions. This has helped the research team to follow up and ensure that follow through on consents are completed. Keitrina will finish at Miami Cancer Institute in October 2023. A new student from Florida International University is due to join the research team and participate in this research study, beginning in October/November 2023.

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

Summary: The research team has made significant progress in examining the treatment effect of Zika virus on the pediatric brain tumor, diffuse intrinsic pontine glioma (DIPG). The research team has determined that in DIPG, the protein, cluster of differentiation 24 (CD24), is not required to enter the cell as it is required for neuroblastoma. This may relate to the transformed nature of the DIPG cells and point to an additional mechanism for the Zika viral specificity for DIPG. The laboratory is currently investigating novel targets that allow the Zika virus to target DIPG. Zika virus is an effective therapy in mice engrafted with DIPG subcutaneously and demonstrates a greater than 95% reduction in DIPG tumor size without tumor regrowth. This is

significant because for the last 20 years, there have been no advances in DIPG treatment that changes the overall dismal survival. DIPG claims the life of children normally within the first year after diagnosis. The tumor microenvironment is known to be significant in cancer treatment. As a result, the research team is currently expanding these results in subcutaneous tumors to mouse intracranial tumors. Mouse models and tumor sample acquisition have been challenging for DIPG. Through collaboration with a Nemours team in Delaware, the research team has successfully grown DIPG cells in the mouse brain and sustained the tumor with no effects to the mouse for two weeks. The tumor cells are tagged with a bioluminescence marker to allow for quantitative imaging and future evaluation when treated with Zika virus. Zika virus will be directly delivered to the DIPG tumor within the mouse brain to treat tumor. This delivery mechanism will recapitulate the subcutaneous DIPG tumor work completed by the research team. Moreover, the direct delivery mechanism has been successfully completed in other laboratories studying a related tumor, glioblastoma. This exciting research is an incremental step forward for children with DIPG, which is fatal. This work offers hope to Florida children and their families who are impacted by DIPG.

Follow on Funding: None at the time of reporting.

Collaborations: Dr. Sigrid Langhans' laboratory at Nemours Children's Hospital, DE is a collaborator to this research project

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

Summary: Glucocorticoids (GC) are a major component of therapy of pediatric acute lymphoblastic leukemia (ALL). ALL relapse is associated with mutations/deletion of NR3C1 (glucocorticoid receptor, GR), NR3C2 (mineralocorticoid receptor- MR) and NSD2 (a histone methyltransferase). These mutations may affect therapeutic response to GC. The researchers characterized a mutation of NSD2 in relapsed childhood ALL using cell lines and patient specimens. NSD2 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 allowed the nuclear receptor subfamily 3 group C member 1 gene to again be activated by GC treatment, leading to higher levels of the GR that could then carry out a transcriptional program that kills ALL cells through induction of the pro-apoptotic BIM protein. Major findings includes a pan histone deacetylase inhibitor vorinostat did not affect the viability of ALL cells nor increased activity of GC. A histone deacetylase 3 (HDAC3) specific inhibitor inhibited growth of NSD2 mutant cells but did not increase GC sensitivity. A G9a inhibitor enhanced GC sensitivity of NSD2 mutant cells and inhibited growth of NSD2 mutant cells on its own, representing a potential new therapeutic strategy. The researchers found that enhancer of zeste homolog 2 (EZH2) inhibitors could enhance the effects of GC in some NSD2 wild-type ALL cell lines which did not have mineralocorticoid receptor (MR) and glucocorticoid receptor (GR). gene deletions. This was associated with increase expression of the pro-apoptotic BIM

protein. The researchers obtained several new NSD2 inhibitors. These decrease H3K36me2 levels in ALL as well as myeloma and mantle cell lymphoma cells. NSD2 inhibitors selectively decreased the viability of NSD2 mutant ALL cells. NSD2 inhibitor pretreatment led to increased expression of the GR and restored GC sensitivity of NSD2 mutant ALL cells. In collaboration, the researchers characterized the effects of novel NSD2 degraders which bind to the N-terminal PWWP domain of NSD2, and link the protein to the ubiquitin ligase machinery, targeting NSd2 for degradation by the proteosome.The NSD2 degrader eliminated NSD2 expression in mutant and wild-type ALL cell lines. The NSD2 degrader compound was more toxic to NSD2 mutant ALL cell lines. In response to NSD2 degrader, GR level rose in NSD2 mutant ALL cells and the cells became more sensitive to GC, indicating that this new agent could be a therapeutic agent for NSd2 mutant ALL. The researchers developed multi-omic datasets in NSD2 mutant and wild-type ALL cells using the newer CUT and RUN technology to fully understand how mutant NSD2 creates aberrant patterns of gene expression and represses the expression of the NR3C1 gene encoding the GR.

Follow on Funding: Licht, JD, PI. Myeloma Solutions Fund. 09/01/2023-08/31/2026. Total Amount Awarded: $1,000,000.

Collaborations: There is a collaboration with Jun Qi- Dana-Farber Cancer Institute Harvard University, who designed a NSD2 inhibitor for further treatment in the ALL-cell lines. There is also a collaboration with Cheryl Arrowsmith from University of Toronto) and Lindsey Ingerman James from University of North Carolina at Chapel Hill, who designed this NSD2 degrader for further treatment in the ALL-cell lines.

Journals: Morozov VM, Riva A, Sarwar S, et al. HIRA-mediated loading of histone variant H3.3 controls androgen-induced transcription by regulation of AR/BRD4 complex assembly at enhancers, *Nucleic Acids Research*, 2023;, gkad700, doi:10.1093/nar/gkad700.

Hanley RP, Nie DY, Tabor JR, et al. Discovery of a Potent and Selective Targeted NSD2 Degrader for the Reduction of H3K36me2. J Am Chem Soc. 2023;145(14):8176-8188. doi:10.1021/jacs.3c01421.

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

Summary: The survival rates of pediatric cancer patients have greatly increased over the last decades with advances in technology and improvements in multimodal therapy and combination chemotherapies. While the overall five-year relative survival for childhood cancer from 2012-2018 was 85%, children with high-risk tumors, metastatic disease at diagnosis, or those with progressive, refractory, or relapsed disease still have only modest improvement and many a dismal prognosis. The goal of this project is to develop clinically translatable Natural Killer (NK) cell-based immunotherapeutic strategies that can increase the response rate and lower the relapse rate in these high-risk pediatric patients. To achieve this, researchers have proposed to use NK cells activated with particle technology (PM21-NK cells). Although highly cytotoxic,

PM21-NK cells express inhibitory receptors that compete for ligands that trigger NK cell lysis, such as PVR, a receptor highly expressed in neuroblastoma. The researchers propose to eliminate expression of PVR inhibitory receptors in PM21-NK cells to further enhance cytotoxicity and prevent exhaustion. In year one of this project, researchers have acquired 11 different neuroblastoma cell lines representing different features known to be present in high-risk neuroblastoma. Four of these cell lines have so far been cultured and characterized to be used to determine what combinations of gene editing in PM21-NK cells enhance anti-tumor functions across different neuroblastoma models. Initial testing using the SK-N-As cell line has shown that each receptor as a single knockout improved NK cell cytotoxicity, with T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT) knockout (KO) followed by poliovirus receptor-related immunoglobulin domain-containing (PVRIG) KO having the most profound effect. Double knockout of TIGIT and PVRIG outperformed single knockouts of those receptors, with triple knockout of TIGIT, PVRIG, and CD96 incrementally improved cell killing. These preliminary results were presented on two posters at the American Association for Cancer Research (AACR) 2023 national conference. Also, in the first year of this project one neuroblastoma patient sample was received. NK cells were isolated from the blood, expanded and cryopreserved as well tumor tissue was biobanked for future use in this study. Methods for tumor organoid culture and ex vivo proliferation of patient-derived tumor are under establishment in the laboratory and will enable better representation of the patient tumor heterogeneity and establish the key molecules that impede NK-cell function and test appropriate treatment combinations to maximize NK cell infiltration and anti-tumor response. Collectively, these results and progress thus far have set the stage to begin testing the innovative NK cell-based therapeutic combinations that have the potential to increase the response rate and lower the relapse rate in pediatric neuroblastoma patients and are a new and exciting avenue to explore for positive effects on the health of an important vulnerable population of Floridians.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Hasan MF, Croom-Perez T, Oyer J, et al, 1009 Knockout of the inhibitory receptor TIGIT enhances anti-tumor response of ex vivo expanded NK cells and prevents fratricide with therapeutic Fc-competent TIGIT antibodies. *Journal for ImmunoTherapy of Cancer* 2022;10. doi:10.1136/jitc-2022-SITC2022.1009.

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

Summary: Pediatric cancer is a leading cause of deaths in children, and there is intense interest in developing novel therapies for treatment of this specialized population of cancer patients. There are major challenges in treatment of many pediatric cancers, since many childhood cancers can differ substantially from that of adult cancers and are often not recognized by the immune system for efficient clearance. The research staff have made significant progress in the first year of this funded project, which is aimed at testing the hypothesis that oncolytic viruses–defined as safe viruses with a specificity for tumor verses

normal tissue–can be used to enhance the ability of immune cells to recognize and kill pediatric cancer cells. The research staff progress has resulted in five different substantial results a peer-reviewed publication on how chemotherapy enhanced oncolytic virus killing of pediatric brain cancer cells, developing a novel experimental system for watching the killing of pediatric cancer cells in live and real time, testing a wide range of pediatric cancer cell lines for their infection by the oncolytic virus called P/V virus and recognition by Natural Killer (NK) cells, identification of two pediatric cell lines (SK-N-SH and RD cells) as excellent model systems to test the above hypothesis, since they show enhanced NK cell killing when infected with oncolytic virus; and generating high resolution data on how the SK-N-SH cells respond to P/V virus infection and what changes in the cells make them more sensitive to killing by NK cells in culture. The research staff are now analyzing thousands of cell genes that change expression after P/V infection and how these changes in genes lead to enhanced killing by NK cells. Because the research staff found changes in expression of neuroblastoma cell genes, the staff tested the hypothesis that chemical anti-cancer drugs would enhance killing of pediatric cancer cells in culture. The publication from the research group (Kedarinath et al., Anti-cancer Drugs, 2023) showed that a commonly used chemotherapy drug (5-Azacytidine) could combine with the oncolytic P/V virus to increase killing of pediatric cancer cells. This is an important result suggesting that oncolytic viruses can be used in combination with other commonly used therapeutic approaches such as chemotherapy. The results from the research staff discoveries are an important step for Floridians, as the data support a novel approach to pediatric cancer therapy–combining oncolytic viruses with chemotherapy or with NK cell immunotherapy. The research staff discoveries form the basis for new clinical approaches to treat pediatric cancer, which is known to be resistant to common approaches used for adult cancers

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Kedarinath K, Shiffer E, and Parks GD. DNA Methyltransferase Inhibitor 5-Azacytidine Enhances Neuroblastoma Cell Lysis by An Oncolytic Parainfluenza Virus. *Anti-Cancer Drugs*. 2023. doi: 10.1097/CAD.

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

Summary: 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 RNA vaccine.

Principal Investigator: Paul Castillo Caro, MD

Organization: University of Florida

Summary: The research team robustly confirmed the findings presented in the original grant proposal. A potent synergy between chimeric antigen receptor(CAR) T cells and ribonucleic acid (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, et al. CAR T Cell Locomotion in Solid Tumor Microenvironment. *Cells*. 2022; 11(12):1974. doi:10.3390/cells11121974.

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

Summary: Hematologic malignancies have previously been identified at the number one priority amongst the Florida BMT and Cell Therapy Consortium (FPBCC) due to poor survival rates. Through retrospective analysis, this disease group demonstrates a low survival in 65% of high risk patients—those with a poor donor source and/or high risk disease. In this group there is a high non-relapse (26%) and relapse (18%) mortality at 12 months time. In order to prioritize treating this disease, this grant has funded prospective, multi-institutional clinical trial utilizing Orca Q Engineered graft in pediatric patients with hematologic malignancies. Since the award started, researchers have been able to open this trial at the University of Florida and enroll a patient with high risk acute myeloid leukemia. This patient is currently >270 days from their haploidentical stem cell transplantation living disease free and without evidence of graft versus host disease nor graft rejection. Investigators from the FPBCC have had monthly meetings devoted to study updates and development, discussion of high risk patients with case presentations. Data collection was performed reviewing a cohort of 155 children with hematologic malignancies treated at participating institutions between 2015 and 2020. These results were presented at the Transplant and Cellular Therapy Meeting in Orlando, FL in February 2023 and a manuscript is currently under development. Over the past year the research team and project staff have been working closely in collaboration with the other

FPBCC programs in order to open the clinical trial at these sites. Nicklaus Children's had their site initiation visit completed July 5th and will begin enrolling patients starting August after receiving electronic data collection training through Orca Biosystems, Inc. Subcontracting is near complete at all sites and researchers are currently awaiting IRB approval for three sites, which are not ceding to the central IRB. The research team anticipates improved and increased enrollment of patients beginning September 2023 as manufacturing of engineered grafts increase and sites the additional sites are open for patient accrual.

Follow on Funding: None at the time of reporting.

Collaborations: Dr. Jolan Walter's laboratory at the University of South Florida in the Pediatric Allergy and Immunology Department has been involved in this project and contracting has completed to perform immune reconstitution studies for patients. Nicklaus Children's Hospital has undergone their site initiation visit and will begin electronic data collection system training in preparation of enrolling patients. Nemours Children's Hospital, Johns Hopkins All Children's Hospital, and University of Miami Children's Hospital are awaiting final contracting and institutional review board (IRB) approval to begin enrolling patients.

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

Summary: 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 research team 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, they 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

Summary: The research team has collaborated to produce 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 meet with the family to discuss the study and consent the parent(s). The research team is recruiting in both the inpatient and outpatient pediatric hematology/oncology settings in Gainesville. The research staff have enrolled 22 families from UF, and three families have completed the counseling and two received their incentive. At University of South Florida (USF)/Tampa General Hospital (TGH), the research staff are in the process of securing intitutional review board (IRB) approval and is projected to receive approval July 2023. The research team has prepared the protocol for recruitment at USF/TGH and visually appealing flyers, which have been submitted the IRB. After IRB approval, the research team will start recruitment of families 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). Due to individual IRB submissions, the researchers have created data access groups (DAGs) for the Unviersity of Florida (UF) and TGH to enroll patients' protected health information PHI separately. 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: None at the time of reporting.

Collaborations: At UF, two medical students are volunteering as interns to help enroll new participants in the clinic and the inpatient setting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix T: Live Like Bella Initiative
Fiscal Year 2022-2023 Active Grants
Funded Fiscal Year 2020-2021

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
21L01	Relinquished						
21L03	University of Florida	Mingyi Xie, PhD	$247,000.00	4/30/24	Yes	No	Yes
21L04	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Uwe Rix, PhD	$247,000.00	4/30/24	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
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	4/30/24	No	No	Yes

1. **Grant#:** 21L03 Target RNAs induce microRNA degradation in apoptotic T-cell acute lymphoblastic leukemia cells

Principal Investigator: Mingyi Xie, PhD

Organization: University of Florida

Summary: Researchers have been conducting a follow-up study revisiting some of the putative target-directed microRNA degradation (TDMD) triggers researchers identified utilizing microribonucleic acid (miRNA)-target chimeric data (CLASH) and a series of stringent overexpression experiments. In the original study, small RNA (sRNA)-seq was used to quantify the absolute abundance of specific miRNAs with and without overexpression of putative TDMD triggers. Using this technique, the analysis suggested that the putative trigger, BCL2L11, was able to induce degradation of miR-221/222. Interestingly, upon further scrutiny, researchers found that original analysis method failed to consider miRNA isoforms (such as single, or multiple non-templated nucleotide extensions) and instead only counted templated (genome-mapping) sequences. When modified to include these miRNA isoforms, were found that BCL2L11 simply induces three extensions of both miR- 221/222, with no significant change in abundance. This phenomenon is well established and is often referred to as target-directed tailing and trimming (TDTT). During TDTT, RNA transcripts that interact with miRNAs using non-canonical base pairing expose the three ends for modification by terminal nucleotidyltransferases (TENTs) and/or terminal uridylyl transferases (TUTs). Recently, three key enzymes (TENT2, TUT4, and TUT7) were found to regulate the vast majority of TDTT in HEK293T. Interestingly, very few targets that induce TDTT have been scrutinized to identify the specific enzymes inducing the modification. One well-known example is the CYRANO TDMD trigger, which induces tailing of miR-7 specifically via TENT2, but this tailing was found not to be required for its concomitant degradation of miR-7. Conversely, in the case of miR-345, individual loss of TENT2, TUT4, TUT7, or both TUT4 and TUT7 failed to oblate tailing. Only when TENT2, in addition to TUT4/7, were knocked out could miR-345 tailing be completely abolished. These independent findings suggest that there are both triggers that recruit specific enzymes, and non-specific triggers that redundantly recruit TENT2, TUT4, and/or TUT7. To decouple the observed TDTT from TDMD and interrogate to what extent these enzymes are involved in the tailing of

miR-221/222 via BCL2L11, researchers have established clonal Kos of TENT2, TUT4, TUT7, and TUT4/7 in HEK293T. In addition, these clones were established in HCT116 to probe the cell line specificity of any possible interactions. Utilizing these cells, researchers transiently overexpressed various triggers, and used northern blotting to observe alterations to tailing induced by the triggers. Presumably, if a trigger is specifically regulated by one of the tailing enzymes, loss of that enzyme should significantly reduce the abundance of tailed isoforms. Unfortunately, researchers did not observe any obvious changes to tailing of miR-221 or miR-218 induced by the triggers. These results suggest the possibility that these triggers are of the non-specific variety and are akin to the triggers that regulate the tailing of miR-345. The TENT2, TUT4/7 triple KO cells are currently in production to probe this question.

Follow on Funding: Xie, M, PI. Exploring microRNA degradation in T-cell acute lymphoblastic leukemia. National Institutes of Health/NCI. 07/15/2023-07/14/2028. Award Notice Date 07/11/2023. Total Amount Funded: $503,845.00. CFDA Code: 396.

Xie, M, PI. Molecular mechanisms for regulating microRNA levels in metazoans. National Institutes of Health/NCI. 08/13/2018-06/30/2028. Award Notice Date: 08/18/2023. Total Amount Awarded: $411,750.00. CFDA Code: 859.

Collaborations: University of Florida is the major site for the proposed research. Tianqi Li, a fifth year Biomedical Sciences PhD candidate, Nicholas Hiers, a third year Biomedical Sciences PhD candidate and Conner Traugot, a second year Genetics and Genomics PhD candidate are involved in the research proposed in this project during the reporting period.

Journals: Sheng, P., Li, L., Li, T. et al. Screening of Drosophila microRNA-degradation sequences reveals Argonaute1 mRNA's role in regulating miR-999. *Nat Commun* 14, 2108 (2023). Doi:10.1038/s41467-023-37819-9.

Patents: None at the time of reporting.

2. **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, Inc.

Summary: 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. Researchers aim to determine the cellular phenotypes affected by PARP16 and its in vivo functional relevance in EWS. Researchers will 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 schlafen family member 11 (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.

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

Principal Investigator: Jonathan Licht, MD

Organization: University of Florida

Summary: The researchers performed a screen to define genes up regulated by mutant nuclear receptor binding SET domain protein 2 (NSD2) that encourage adhesion to cells of the blood brain barrier. The researchers continue to work to validate these genes in secondary assays. The researchers co-cultured ALL cells with endothelial cells of the blood brain barrier. The interaction changes gene expression both in the leukemia cells and the endothelial cells. Genes activated in NSD2 wild-type cells by interaction with the endothelial cells were found to be already increased in expression in NSD2 mutant cells. This may help explain the brain invasive behavior of the mutant ALL cells. Using a program called niche net, the researchers defined signaling pathways affected by leukemia-endothelial cell interaction. For example, IL1B was expressed in NSD2 mutant but not NSD2 wild-type cells. This could affect the permeability of endothelial cells and encourage brain invasion. The explored new anti NSD2 therapies including NSD2 antisense oligonucleotides. Antisense oligonucleotides can eliminate the expression of NSD2, decrease the characteristic H3K36me2 modification carries out by NSD2

and decreased adhesion activity of cells. With the Musco lab in Milan, Italy the laboratory explored the function of the PHD chromatin binding domain within NSD2. Point mutations of this domain inhibit the ability of the NSD2 protein to interact with the repressive histone 3 lysine 27 trimethyl modification, and as such could affect the ability of NSD2 to activate genes. Repletion of NSD2 low cells with NSD2 activated expression of hundreds of genes, notably those involved in adhesion and migration. The PHD mutants activated only a subset of these genes. NSD2 stimulated cell adhesion and the NSD2 PHD mutants were defective in stimulating adhesion. Genome wide studies of the epigenome of cells repleted with NSD2 wild-type or NSD2 PHD mutant protein showed that the PHD mutants failed to increased activation associated histone 3 lysine 27 acetylation (H3K27Ac) at genes normally turned on by NSD2 and failed to decrease. Levels of repressive H3K27me3 at these genes. This is consistent with a defect in sensing H3K27me3 by the mutant forms of NSD2. The promoter gene regulatory regions of genes activated by wild type NSD2 but not activated by mutant NSD2 differed suggesting that the NSD2 PHD domains are particularly important for collaboration with specific transcriptional regulator factors at gene promoters. These latter findings are being finalized for publication.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Lan B. Hoang-Minh, PhD

Organization: University of Florida

Summary: 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 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. PD-1 inhibitors have been used successfully in the clinic against multiple solid cancers, particularly when given before surgical tumor removal. These studies show that anti-PD-1 treatment before (neoadjuvant) and after (adjuvant) surgery enhances survival time in a preclinical resection model of recurrent glioma that the researchers have established when compared with adjuvant treatment alone.

Researchers have found significant differences in signaling pathways that regulate the activation and function of immune cells, including T cells, B cells, natural killer cells, and macrophages and immune cell survival, proliferation, and differentiation between neoadjuvant and adjuvant treatment groups. Genes involved in regulating the recruitment of other immune cells, such as monocytes, T cells, and NK cells, were also upregulated in the neoadjuvant treatment group when compared with the adjuvant treatment group. Marker of exhaustion and activation were also differentially expressed on T cells between neoadjuvant and adjuvant groups. Additionally, in collaboration with the University of Florida College of Engineering, the researchers were able to visualize ACT T cells over time after tagging them with optimized nanoparticles and using magnetic particle imaging, a novel non-invasive imaging technology for which human scanners are being rapidly developed. These research findings hold great promise for translating into novel, safer, and highly effective immunotherapy approaches targeting pediatric brain tumors in the clinical setting. This has the potential to significantly enhance and prolong the lives of young patients grappling with these exceptionally aggressive cancer forms and, possibly, offer breakthroughs for other cancer types as well.

Follow on Funding: None at the time of reporting.

Collaborations: University of Florida College of Engineering, Department of Chemical Engineering, Gainesville, Florida, PI: Carlos Rinaldi, Angelie Rivera-Rodriguez, Bo Yu, Hayden Good, and Sitong Liu

Journals: None at the time of reporting.

Patents: None at the time of reporting.

5. **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

Summary: 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, soft tissue sarcomas, among others) while focusing 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 (e.g., diabetes and smoking) on cancer 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 the 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 Department of Vital Statistics birth certificate data. The Data User Agreement and Project Linkage have been updated by the researchers but are still pending approval by the Vital Statistics Department at Florida Department of Health. Considering the continued non-release of requested data, development of this project has not progressed as fast as the research team would like. Despite this major setback, the team has begun childhood cancer research with data from other sources. In collaboration with other

researchers from the University of Miami Miller School of Medicine and Sylvester Comprehensive Cancer Center, research staff evaluated FCDS data on Ewing Sarcoma in Florida, specifically characterizing disparities across race and ethnicity. An abstracted was developed entitled, “Health Disparities Among Patients with Ewing Sarcoma in Florida.” This abstract was presented at the 2023 American Society of Clinical Oncology Annual Meeting, in Chicago, Illinois. This abstract has also been accepted for presentation at the Connective Tissue Oncology Society 2023 Annual Meeting on November 1-4, 2023, and for Gilibend, University of Miami internal conference.

Follow on Funding: None at the time of reporting.

Collaborations: Collaboration between the University of Miami (UM)-Department of Public Health Sciences (Miami, FL) and University of Florida (UF)-Division of Pediatric Hematology/Oncology (Gainesville, FL) is ongoing.Identified in July 2021, Ms. Qinran Liu, a third-year doctoral student in the Epidemiology program at UM continues as part of the study team.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

6. **Grant#:** 21L09 Measuring the effects of brain radiotherapy on scholastic outcome

Principal Investigator: Raymond Mailhot, MD, PhD

Organization: University of Florida

Summary: 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 Intelligence Quotient (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.

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

Principal Investigator: Q.X. Amy Sang, PhD

Organization: Florida State University

Summary: Human pediatric brain cancer is one of the most lethal types of cancer that occur in childhood. A highly efficacious and specific therapy that maximizes the killing power against cancer cells and minimizes the adverse effects on normal cells does not exist for pediatric brain cancer patients. The human brain tumor microenvironment (TME) consists of cancer and noncancerous cells, Stromal cells include endothelial cells, fibroblasts, pericytes, and immune cells, whereas astrocytes, microglia, oligodendrocytes, and neurons make up the tissue-resident population of the brain. TME heterogeneity can protect cancer cells from both immune surveillance and therapeutic treatments. Hence, investigating the TME's effect on cancer cells and exploiting other methods such as immunotherapies has become essential for brain cancer therapy. This project is building novel 3-dimensional (3-D) human pediatric brain cancer models using deadly atypical teratoid/rhabdoid tumor (ATRT), diffuse intrinsic pontine glioma (DIPG), and other pediatric brain cancer cell lines with the incorporation of astrocytes and microglia that are derived from human induced pluripotent stem cells (hiPSCs). The hypothesis to be tested is that the tumor-associated astrocytes may produce human interleukin-4 and other cytokines to polarize tumor-associated microglia, which will produce insulin-like growth factor 1 and other cytokines to promote tumor progression, invasion, and chemoresistance. The first part of the project tests the role of astrocytes and microglia in tumor progression and chemoresistance. While the second tests the ability of both natural killer (NK) and gamma delta-T (GD-T) cells to kill human pediatric brain cancer cells. NK cells and GD-T cells are part of the innate immune system, and they express antitumor activity without the need for human leukocytes antigen matching and prior antigen exposure. Natural killer cells and gamma delta T cells are derived from hiPSCs and used to treat the 3-D cancer cell spheroids or organoids in the presence or absence of astrocytes and microglia to evaluate the killing power at different ratio of T cells to cancer cells to overcome immunosuppression. The research team studied the cytotoxic potential of human iPSCs-derived NK cells and compared it with the cytotoxicity of the human NK-92mi cell line. To compare the killing potential of human iPSCs-derived NK cells and NK92-mi cell line, two types of ATRT cell lines, CHLA02 and CHLA05 were used as target cells. The research staff also investigated the effect of astrocytes on the cytotoxicity of immune cells targeting the ATRT cell lines and studied the proliferative, migratory, and invasive properties of human ATRT and glioma cells. Moreover, the team evaluated the effects of astrocytes on the proliferation, invasion, and migration of various pediatric brain tumor cells. The team compared various conditions of cell cultures to test the invasiveness of human malignant pediatric cancer

cells. The research team is analyzing the results and preparing a manuscript to be submitted for publication in the near future. The team is planning to prepare and submit new grant applications to obtain funding to support this important pediatric cancer research project.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Hua T, Zeng Z, Chen J, Xue Y, Li Y, Sang Q. Human Malignant Rhabdoid Tumor Antigens as Biomarkers and Potential Therapeutic Targets. *Cancers (Basel)*. 2022;14(15):3685. Published 2022 Jul 28. Doi:10.3390/cancers14153685.

Patents: None at the time of reporting.

Appendix U: Live Like Bella Initiative
Fiscal Year 2022-2023 Active Grants
Funded Fiscal Year 2019-2020

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
20L02	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Damon Reed, MD	$787,272.00	4/30/24	No	No	No
20L05	University of Central Florida	Cristina M. Fernandez-Valle, PhD	$218,572.00	11/30/23	No	No	No
20L06	University of Central Florida	Li-Mei Chen, MD, PhD	$109,569.00	11/30/23	Yes	No	Yes
20L07	University of Florida	Elias J. Sayour, MD, PhD	$788,897.00	11/30/23	No	No	No
20L08	University of Florida	Coy Heldermon, MD, PhD	$219,138.00	11/30/23	No	No	No

1. **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, Inc.

Summary: Moffitt Cancer Center has activated the trial here at the center and has sent out information to all affiliate sites throughout the sunshine project network towards opening this study throughout Florida on this grant and throughout the nationwith other foundation funds including from the National Pediatric Cancer Foundation. A total of 17 sites have been activated and are open to enrollment. (four sites in Florida and 13 sites outside of Florida. A total of nine patients have been accrued on this multi-arm trial. Three patients enrolled on Arm A (first strike). Six patients enrolled on Arm B (maintenance). It is too early to report any outcomes at this time. The Principal Investigator role has been transferred from Dr. Damon Reed to Dr. Jonathan Metts. The change in key personnel form was submitted and approved , effective 7/1/23

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. The research staff are pleased to report that progress has been made at all sites towards opening this trial in these early days. Activated Florida Sites:Moffitt Cancer Center, Johns Hopkins All Children's Hospital, University of Florida, University of Miami. Pending Activation Florida Sites: Nemours Orlando: pending IRS approval. A revised resubmission of P01 Grant entitled "Evolutionary Therapy" will be submitted in January 2024 to further these concepts to osteosarcoma and Ewing sarcoma.

Journals: Reed DR, Metts J, Pressley M, et al. An evolutionary framework for treating pediatric sarcomas. *Cancer*. 2020;126(11):2577-2587. Doi:10.1002/cncr.32777.

Patents: None at the time of reporting.

2. **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

Summary: Children born with Neurofibromatosis Type 1 caused by specific pathogenic variants in the neurofibromin gene develop plexiform neurofibromas that carry a high risk of becoming malignant. This type of tumor is then called a malignant peripheral nerve sheath tumor (MPNST) and is the primary reason for death of children with Neurofibromatosis Type 1. To date, there is no blood test to determine if a plexiform neurofibroma has become malignant. Diagnosis is not made until the child complains of increased pain and/or rapid growth of an externally visible tumor is noticed. Children suspected of developing a MPST then undergo repeated magnetic resonance imaging under full anesthesia. Aggressive surgery when possible is conducted followed by chemotherapy. The five year survival is low. The goal of this work is to create a blood test that can be incorporated into routine annual exams as a non-invasive surveillance assay. After obtaining and screening eleven (11) human MPNST cell lines researchers identified a consistently expressed receptor for use as the capture antibody that is not expressed on blood cell type. This year the research team succeeded in creating a novel assay using an antibody to the receptor to capture MPNST cells added to normal human blood. Research staff have tested it on blood of normal human subjects and from four children with NF1 who were diagnosed and treated for MPNST. The future direction is to test the assay using blood from newly diagnosed MPNST patients before and after treatment. The assay will then be tested for use as a surveillance assay. Additional funding will be needed to confirm usefulness and seek protection for its commercial development.

Follow on Funding: None at the time of reporting.

Collaborations: Students trained this year: are Haley Hardin Grad student and Anna Hagel, PhD post-doc

Journals: None at the time of reporting.

Patents: None at the time of reporting.

3. **Grant#:** 20L06 Exosome-mediated activation of matriptase targeting B cell lymphoma

Principal Investigator: Li-Mei Chen, MD, PhD

Organization: University of Central Florida

Summary: Therapies targeting B cell lymphomas include antibody-directed depletion of B cancer cells. Examples are Rituximab targeting cluster of differentiate 20 (CD20) on B cell surface to remove aberrant B cells, inebilizumab (MEDI-551) targeting CD19 on B cells to induce cytotoxicity, or Epratuzumab targeting CD22 to trigger signaling pathways affecting B cell proliferation. Other therapeutics are also in development aiming to interfere with survival factors or mediators involved in B cell functions. Despite the progress made in the immunotherapies, the choice of treatments in non-Hodgkin lymphoma (NHL) is still a challenge in patients with recurrent disease or refractory to the first-line chemotherapy. Alternative options for treating

NHL could help patients who are not responding to the existing treatments. This funded project is to seek a novel method of changing B cancer cell progression trajectory or depleting B cancer cells. A serine protease cascade involving prostasin and matriptase was assembled at the cell surface of B lymphoma cells. Both serine proteases are important players in solid tumor initiation and progression with prostasin being the tumor suppressor and matriptase the oncoportein. Matriptase was reported to be overexpressed in Burkitt lymphoma. In this project, removal of matriptase from B cancer cells was initiated by applying prostasin exosomes to the cancer cells. Exosomes are small naturally occurring membrane vesicles and released by all types of cells in a human body. In recent years, exosomes are being explored as biomarkers and drugdelivery vehicles in disease diagnosis and treatment. A series of experiments were performed to evaluate the B cancer cells' properties including proliferation, apoptosis, migration and invasion in the co-cultures of prostasin exosome and B lymphoma cells. The results indicated that in the presence of prostasin, matriptase is activated and shed off from the cancer cells. The activated prostasin-matriptase cascade reduced B cancer cell proliferation, increased apoptosis and inhibited the cancer cell migration. The shed matriptase accumulated in the extracellular space could modify the surrounding cell matrix via matriptase gelatinase activity. These results suggest that prostasin exosomes can be used as a tool to assemble and initiate the prostasin-matriptase protease cascade in the recipient cells to affect their cellular functions. In future studies, this method can be evaluated in vivo using an animal model. The efficacy and the long-term effectiveness of the prostasin-matriptase protease cascade in cancer cells could be the basis for developing alternative methods in treating B lymphoma.

Follow on Funding: Chen, LM, PI. Becton, Dickinson and Company. Azure Lifesciences Research Reagents Funding Initiative. 08/05/2022-08/04/2023. Total Funds Awarded: $5,000.

Collaborations: None at the time of reporting.

Journals: Manuscript in review: Chen LM, Chai KX. Exosome-mediated activation of the prostasin-matriptase serine protease cascade in Blymphoma. Cancers-2496948, 2023.

Patents: None at the time of reporting.

4. **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

Summary: Under the United States Food and Drug Administration Investigational New Drug Program (FDA-IND) (BB-19304), the researchers conducted a first-in-human (NCT04573140) phase 0 (acceleration titration design) dose study in three patients evaluating the feasibility, safety, and activity of RNA-LPA targeting pp65 messenger ribonucleic acid (mRNA) (tumor associated antigen expressed in glioblastoma23-27) and autologous tumor mRNA in patients with primary MGMT unmethylated glioblastoma. After surgery and chemoradiation, patients received up to three biweekly (every two weeks) doses of RNA- Lipoprotein(a) (LPA) followed by a monthly booster. Patients were treated with mRNA infusion doses of 0.625-1.5 µg/kg. In all patients, the researchers observed significant and rapid increases in pro-inflammatory cytokines and chemokines. All patients developed immune-related symptoms 2-6h post-infusion (e.g. low-grade fever, nausea, chills, rigors) that defervesced within 24-48h. Cytokine release correlated

with mobilization of PBMCs beginning with monocytes at hour 2, followed by lymphocyte nadir and neutrophilia at hour 6. Using multi-parameter flow cytometry, the researchers demonstrate that antigen presenting cells (HLA-DR expressing) rapidly decrease from peripheral blood suggesting recruitment to lymphoreticular organs. While CD11c cells rapidly decrease, there is an increase in: percent circulating plasmacytoid DCs, early activation of CD8 lymphocytes, and expansion of antigen specific T cell responses. Using sequencing of T cell receptors pre- and post-infusion, the researchers show that TCR repertoire of the most prevalent clonotypes changes by ~10% in Patients B42 and E35 after one to two infusions and nearly 20% in Patient A25 after four infusions highlighting the ability of RNA-LPAs to reprogram adaptive immune repertoires. Patients A25 and E35 had increased progression free survivorship compared with historical median of six mo28 and patient B42 has begun long-term follow-up.

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#:** 20L08 Novel immunologic therapy of soft tissue sarcoma

Principal Investigator: Coy Heldermon, MD, PhD

Organization: University of Florida

Summary: 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 Principle 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 they 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: None at the time of reporting.

Journals: None at the time of reporting.

Patents: The research team has contacted the UF intellectual property office to begin the process of evaluation of the isograft lines to patent/license for use as a sarcoma model for therapy development studies.

Appendix V: Live Like Bella Initiative
Fiscal Year 2022-2023 Closed Grants
Funded Fiscal Year 2020-2021

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
21L08	University of Miami	Regina Graham, PhD	$247,000.00	6/30/23	No	No	Yes
21L11	University of Central Florida	Annette R. Khaled, PhD	$123,500.00	9/30/22	Yes	Yes	Yes

1. **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

Summary: The goal of this work is to develop gadolinium containing carbon nitride dots (CNDs) for bio-imaging and tumor targeted drug delivery. Researchers recently demonstrated that gadolinium (Gd)-CNDs synthesized using gadolinium trichloride (GdCl3) appear be a better contrast agent than Gd-CNDs synthesized from gadopentetic acid ((Gd-DTPA) as well as gadopentetic acid, commercially known as Magnevist, itself. In addition, researchers have shown that the Gd-CNDs synthesized from GdCl3 demonstrated tumor cell specificity compared to the Gd-CNDs synthesized using Gadopentetic acid. Furthermore, the GdCl3 synthesized CNDs were relatively non-toxic to cells even after a 72-hour exposure. Conversely toxicity was observed with the Gd-CNDs synthesized using Gd-DTPA in a dose dependent manner. The toxicity was greater in the non-tumor (A) verse tumor (B) cell line. Researchers are currently repeating the synthesis to confirm results and also plan to conjugate a fluorescent tag onto the CNDs. The fluorescent molecule researchers will use is one the research group developed. The resulting CD-fluorescent molecule was very bright and easy to visualize in cells. It is a small molecule and in previous experiments did not affect cell uptake or selectivity. The researchers have identified additional ways to target carbon dots to brain tumors and plan to explore these in the future.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Vallejo FA, Sigdel G, Veliz EA, et al. Carbon Dots in Treatment of Pediatric Brain Tumors: Past, Present, and Future Directions. Int J Mol Sci. 2023 May 31;24(11):9562. Doi:10.3390/ijms24119562.

Cilingir, EK, Hettiarachchi, SD, Zhou, Y, et al. Carbon Dot Nanoarchitectonics: Quadruple conjugated nano model for superior cancer cell-nucleus targeting and multi drug delivery. SSRN. July 8, 2022. Doi:10.2139/ssrn.4151704.

Domena JB, Ferreira BCLB, Cilingir EK, et al. Advancing glioblastoma imaging: Exploring the potential of organic fluorophore-based red emissive carbon dots. J Colloid Interface Sci. 2023 Nov 15;650(Pt B):1619-1637. Doi:10.1016/j.jcis.2023.07.107. Epub 2023 Jul 21.

Patents: None at the time of reporting.

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

Principal Investigator: Annette R. Khaled, PhD

Organization: University of Central Florida

Summary: 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 two manuscripts and a patent application filed for the use of CCT as a diagnostic marker for cancer.

Follow on Funding: Khaled, AR, PI. United States Department of Defense. Peer Reviewed Cancer Research Program (PRCRP) Idea. 10/01/2023-09/30/2025 $592,920 award Submitted 8/7/2022.

Collaborations: None at the time of reporting.

Journals: Cox A, Nierenberg D, Camargo O, et al. Chaperonin containing TCP-1 (CCT/TriC) is a novel therapeutic and diagnostic target for neuroblastoma. Front Oncol. 2022;12:975088. Published 2022 Sep 15. Doi:10.3389/fonc.2022.975088.

Ghozlan H, Cox A, Nierenberg D, King S, Khaled AR. The TriCky Business of Protein Folding in Health and Disease. Front Cell Dev Biol. 2022;10:906530. Published 2022 May 5. Doi:10.3389/fcell.2022.906530.

Patents: CHAPERONIN-CONTAINING TCP-1 INHIBITORS FOR THE TREATMENT OF CANCER. For which an application for U.S. Letters Patent was filed on March 5, 2021 as Serial No. 63/157,051. Converted on March 5, 2022.

Appendix W: Live Like Bella Initiative
Fiscal Year 2022-2023 Closed Grants
Funded Fiscal Year 2019-2020

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
20L01	Florida State University	Akash Gunjan, PhD	$219,138.00	5/31/23	Yes	No	Yes
20L03	H. Lee Moffitt Cancer Center and Research Institute, Inc.	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	Yes
20L09	University of Miami	Julio Barrredo, MD	$219,138.00	11/30/22	No	No	Yes

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

Summary: Deoxyribonucleic acid (DNA) is human genetic material, and it regulates all aspects of human health, including diseases such as cancer. Histones are proteins that bind 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 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. 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, the research team 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 (α-KG). High levels of α-KG in turn drive the excessive removal of a chemical modification known as "methylation" from DNA and histone proteins, resulting in very low levels of methylation in the H3.3K27M 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 performed genomic sequencing to understand the effects of IDH1 inhibitors at the molecular

level. The researchers are 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.

Follow on Funding: Gunjan, A, PI. Combination therapeutics for H3 mutant childhood brain tumors. Live Like Bella Pediatric Cancer Research Initiative. 04/01/2024-3/31/2027. Amount Requested: $250,000. Pending.

Collaborations: None at the time of reporting.

Journals: Phillips EON, Gunjan A. Histone variants: The unsung guardians of the genome. DNA Repair (Amst). 2022;112:103301. Doi:10.1016/j.dnarep.2022.103301.

Huang XW, Cheng XR, Wang N, et al. Histone variant H3.3 and its functions in reprogramming. Yi Chuan. 2018;40(3):186-196. Doi:10.16288/j.yczz.17-233.

Patents: None at the time of reporting.

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

Principal Investigator: Bijal Shah, MD, MS

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: Pediatric Burkittlymphoma (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 the 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 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 study described the dependence of PEBL on the anti-apoptotic protein MCL-1 and highlighted the relationship between MYC and the protein MCL-1. The study demonstrated that changes in MYC were associated with commensurate changes in MCL-1. The researchers further demonstrated that targeting MCL-1 directly could kill BL cell lines. Notably, it was also demonstrated that doxorubicin, a key chemotherapeutic component in PEBL and adult BL regimens, could act in part by suppressing MCL-1. The researchers 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. The researchers 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 to new co-investigators. 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, the analyses did not see an association with increases in STAT1 or STAT3 signaling. The researchers went on to show 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, it was found 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. 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.

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

Principal Investigator: Tamarah Westmoreland, MD, PhD

Organization: The Nemours Foundation

Summary: This grant to address the treatment of Cisplatin resistant neuroblastoma with the oncolytic Zika virus (ZIKV) has been very successful. The research team has met the aims of the grant demonstrating that Zika virus is able to eradicate Cisplatin resistant neuroblastoma both in cell culture and in vivo. The research team will be applying for additional funding to build on this research. This grant demonstrated that Zika virus is capable of overcoming the Cisplatin resistance in neuroblastoma and lead to a survival advantage with treatment. The future grant will focus on multidrug resistant neuroblastoma treatment with Zika virus as well as incorporating Zika virus treatment within the current COG protocol for high-risk neuroblastoma and recurrent neuroblastoma.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: American Pediatric Surgical Association 2022 Talk
Zika Virus as an Oncolytic Treatment for High-Risk Cisplatin-Resistant Neuroblastoma
Joseph Mazar, Jeanne Brooks, Matt Peloquin and Tamarah J. Westmoreland
Nemours Children's Hospital, 6535 Nemours Parkway

Patents: None at the time of reporting.

4. **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

Summary: In the present study, the research staff uncovered the transcription factor (TAF1) as a novel adenosine 5 monophosphate-activated protein kinase alpha2 (AMPKα) substrate within a putative multi-protein chromatin-associated complex that, following AMPK's TAF1 phosphorylation at Ser1353, downregulates histone gene transcription, among others, in ALL cells in response to energy/metabolic stress. As strong evidence of this model, AMPKα2 or/and TAF1 knockdown/knockout in ALL cells downregulated histone mRNA. To common knowledge, this non-canonical function of AMPK that phosphorylates TAF1 to regulate histone gene expression in response energy/metabolic stress has not been previously described. Although this study primarily focused on AMPKα2, researchers also uncover AMPKα1 localization to the nucleus in ALL cells suggesting a similar role for AMPKα1 following its nuclear translocation. The latter is important because some subtypes of ALL express low to undetectable levels of AMPKα2. Importantly, the clinical relevance of the experimental model findings was confirmed in primary ALL patient cells and in vivo in NSG mice. Further elucidation of the physiological effects of AMPK's interactions to chromatin-associated proteins elicited by energy/metabolic stress will provide valuable biological insight and confirmation of an epigenetic role for AMPK. In conclusion, researchers uncovered a novel non-canonical function of AMPKα2 that phosphorylates and regulates TAF1 function, both members of a putative chromatin-associated transcription complex to regulate histone gene expression, among others, inducing adaptive transcriptome changes in response to alterations in cellular energy status in ALL. Fully delineating the networks by which AMPK regulates adaptive responses to energy/metabolic stress, either via epigenetic mechanisms or other, will allow the rationale development of strategies to overcome de novo or acquired resistance in ALL and possibly other cancers.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Sun, G, Leclerc, G, Chahar, S, Barredo, J. AMPK Associates with Chromatin and Phosphorylates the TAF-1 Subunit of the Transcription Initiation Complex to Regulate Histone Gene Expression in ALL Cells. Mol Cancer Res 2023; doi:10.1158/1541-7786.MCR-23-0502.

Patents: None at the time of reporting.

Appendix X: Live Like Bella Initiative
Fiscal Year 2022-2023 Closed Grants
Funded Fiscal Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
9LA01	Florida State University	Amy Q. Sang, PhD	$250,000.00	3/31/22	No	No	Yes
9LA02	H. Lee Moffitt Cancer Center and Research Institute, Inc.	Mihaela Druta, MD	$784,733.00	9/30/23	No	No	No

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

Principal Investigator: Amy Q. Sang, PhD

Organization: Florida State University

Summary: 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. This funded project has built a novel 3-dimensional spheroid model that mimics human pediatric brain rhabdoid tumor formation. The state-of-art Clustered regularly interspaced palindromic repeats (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 SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily B, Member 1 (SMARCB1) tumor suppressor; thus, deleting the SMARCB1 gene in early NPCs may generate a rhabdoid tumor model for therapeutic evaluation. ATRT is characterized by the biallelic inactivation of a tumor suppressor gene SMARCB1 and has a high embryonic gene expression profile. The guide ribonucleic acid (RNA) molecules were designed, and CRISPR-Cas9 gene-editing technology was used to knock out the SMARCB1 gene to mimic human ATRT development in childhood. The gene knockout construct was transfected into induced pluripotent stem cells and experiments were performed to verify if the SMARCB1 gene was knocked out. Deoxyribonucleic acid (DNA) sequencing experiments were carried out to verify the gene knockout, and immunological experiments further verified that the SMARCB1 protein was not produced by the stem cells or the neural spheres. Cell lines with more mutated SMARCB1 expression were characterized for their morphology. They were also being differentiated into spheroids using neural progenitor protocol. Combining the knockout of SMARCB1 with Tp53 led to cancerous development. Several clones were used for further SMARCB1 transfections using the same combination of SMARCB1 and Tp53 targeted CRISPR-Cas9 plasmid. The transfected cells that expressed the markers of interest were collected and replated as single cells. Western blot analysis for these new cell lines showed a low SMARCB1 expression and diminished endogenous control proteins. Research staff generated new SMARCB1 knockout clones and were able to knockdown the SMARCB1 protein expression by around 80%, according to the Western blot results. The research team characterized these clones as well as obtain more clones with SMARCB1 knockout and examining the morphology of these mutated clones and ATRT cell lines. These ATRT-like brain organoids were used to evaluate efficacy of different therapeutic drugs and experimental compounds. Several compounds and their combination treatments were identified to be highly efficacious to inhibit ATRT tumor growth. In summary,

researchers have completed all the specific aims and tasks. Currently researchers are preparing two new original research papers for publication and grant applications for future funding.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Khamis ZI, AI-Akkary N, Hua T, Draughon SA, Li Y, Sang QA Clinical investigations of immunotherapy for human primary brain tumors. *Neuroimmunol Neuroinflammation*. 2020 October; 7. Doi: http:l/dx.doi.org/10.20517/2347- 8659.2020.43.

Hua TT, Bejoy J, Song L, Wang Z, Zeng Z, Zhou Y, Li Y, Sang QA Cerebellar Differentiation from Human Stem Cells Through Retinoid, Wnt, and Sonic Hedgehog Pathways. *Tissue Eng Part A* 2021 Jul;27(13-14):881-893. Doi: 10.1089/ten.TEA2020.0135. Epub 2020 Oct 1.

Hua T, Kiran S, Li Y, Sang QA Microplastics exposure affects neural development of human pluripotent stem cell-derived cortical spheroids. *J Hazard Mater*. 2022 Apr 11;435:128884. Doi: 10.1016 .jhazmat.2022.128884.

Patents: None at the time of reporting.

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

Principal Investigator: Mihaela Druta, MD

Organization: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Summary: The purpose of the study is to see if Nivolumab (Dose Level 1) or Nivolumab in combination with Azacitidine (Dose Levels 2 and 3) given to patients before and after surgery is safe and to see if patients are able to successfully complete the treatment before their surgery without any extended delays in treatment. As of June 30, 2022, a total of 16 patients have been accrued (one replaced due to withdrawal prior to treatment and one will be replaced due to disease progression during the 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 3rd 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 2nd patient experienced a DLT and the 3rd patient had disease progression during their first week of treatment so will be replaced. Based on how the new 3rd 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.

Appendix Y: Live Like Bella Initiative
Fiscal Year 2022-2023 Closed Grants
Funded Fiscal Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	End Date	Follow-on Funding	Patents	Publications
8LA05	Florida International University	Diana Azzam, PhD	$700,000.00	3/31/22	No	No	Yes

1. **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

Summary: 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 ten 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, et al. Clinical Utility of Functional Precision Medicine in the Management of Recurrent/Relapsed Childhood Rhabdomyosarcoma. *JCO Precis Oncol*. 2021;5:PO.20.00438. Published 2021 Oct 27. doi:10.1200/PO.20.00438.

Patents: None at the time of reporting.