Alzheimer's Disease Research Grant Advisory Board

Ed and Ethel Moore Alzheimer's Disease Research Program

2019-2020 Report

Ron DeSantis
Governor

Scott Rivkees, MD
State Surgeon General

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ED AND ETHEL MOORE ALZHEIMER'S DISEASE RESEARCH PROGRAM
INTRODUCTION AND OVERVIEW

Alzheimer's disease is a debilitating brain disease that affects approximately 5.7 million Americans, including 540,000 Floridians, over the age of 65.[4] It is estimated that by 2025, over 720,000 seniors will be living with this disabling disease in the state of Florida.[1] Alzheimer's disease is the sixth leading cause of death in Florida. Additionally, in 2017 it is estimated that 13.5% of Floridians over the age of 65 are likely to have Alzheimer's disease.

According to the National Institute on Aging, a subdivision of the National Institutes of Health, Alzheimer's disease is characterized as an "irreversible, progressive brain disorder that slowly destroys memory and thinking skills, and, eventually, the ability to carry out the simplest tasks."[1] It is the most common cause of dementia among the senior population, with symptoms interfering with normal daily life activities, including loss of thinking, memory, and reasoning abilities. African Americans are twice as likely and Hispanics are one and a half times as likely as older whites to have Alzheimer's disease and other dementias.[2,3,7,8] The prevalence is also higher among women compared to men; two-thirds of Americans with Alzheimer's disease are women.[1] Although there is no known cure, innovative research may provide hope for effective and novel treatment for this incapacitating disease.

To combat these startling statistics, the 2014 Florida Legislature created the Ed and Ethel Moore Alzheimer's Disease Research Program (Program) that was signed and enacted by then -Governor Rick Scott. This Program is managed by the Florida Department of Health. The long-term goals of this Program are to:

a) Improve the health of Floridians by researching improved prevention measures, diagnosis methods, treatments, and cures for Alzheimer's disease.

b) Expand the foundation of knowledge relating to the prevention, diagnosis, treatment, and cure for Alzheimer's disease.

c) Stimulate economic activity in the state in areas related to Alzheimer's disease research.

Annually, the Alzheimer's Disease Research Grant Advisory Board submits a fiscal year progress report by February 15th, as required by section 381.82, Florida Statutes. With the additional reporting requirements resulting from legislative change effective July 1, 2016, this report provides current findings on the return on investment resulting from the state-supported research grant funding.

ALZHEIMER'S DISEASE RESEARCH GRANT ADVISORY BOARD

Ed and Ethel Moore Alzheimer's Disease Research Program Overview

The Ed and Ethel Moore Alzheimer's Disease Research Program was created by the 2014 Florida Legislature and signed by Governor Rick Scott. The purpose of this program in the Florida Department of Health (Department) is to fund research leading to the prevention of, or cure for, Alzheimer's disease. The long-term goals of the program are to:

a) Improve the health of Floridians by researching better prevention, treatments, diagnosis tools, and cures for Alzheimer's disease.
b) Expand the foundation of knowledge relating to the prevention, diagnosis, treatment, and cure of Alzheimer's disease.
c) Stimulate economic activity in the state in areas related to Alzheimer's disease research.

Alzheimer's Disease Research Grant Advisory Board

On October 1, 2014, the State Surgeon General and Secretary of Health appointed 11 members to the Alzheimer's Disease Research Grant Advisory Board (Advisory Board). The Advisory Board authorized in Section 381.82, Florida Statutes, consists of: two gerontologists, two geriatric psychiatrists, two geriatricians, two neuroscientists, and three neurologists.

The Advisory Board advises the State Surgeon General as to the scope of the research program and shall submit its recommendations for proposals to be funded to the State Surgeon General by December 15 of each year. Grants and fellowships shall be awarded by the State Surgeon General, after consultation with the Advisory Board, on the basis of scientific merit. Other responsibilities of the Advisory Board may include, but are not limited to, providing advice on program priorities and emphases; assisting in the development of appropriate linkages to nonacademic entities, such as voluntary organizations, health care delivery institutions, industry, government agencies, and public officials; and developing and providing oversight regarding mechanisms for the dissemination of research results.

Alzheimer's Disease Research Grant Advisory Board Membership

The names and positions of each Alzheimer's Disease Research Grant Advisory Board Member, as of December 12, 2018, are listed below (Biographical Statements or Curriculum Vitae are available upon request):

Gerontologists:

Leilani Doty, PhD, Chair
Associate Director of Programs, Central and North Florida Chapter of Alzheimer's Association

Jacqueline C. Wiltshire, PhD,
Assistant Professor, Health Policy and Management, College of Public Health, University of South Florida

Geriatric Psychiatrists:
Josepha A. Cheong, MD
Professor of Psychiatry and Neurology, University of Florida and Chief, Consult-Liaison Psychiatry, Malcom Randall Veterans Affairs Medical Center

Uma Suryadevara, MD, FAPA
Assistant Professor of Psychiatry and Program Director, Geriatric Psychiatry Fellowship Program, College of Medicine, University of Florida

Geriatricians:
Mariana B. Dangiolo, MD
Assistant Professor of Family Medicine and Geriatrics, College of Medicine, University of Central Florida

Niharika Suchak, MBBS, MHS, FACP
Associate Professor, Department of Geriatrics, College of Medicine, Florida State University

Neuroscientists:
Eunsook Yu Lee, PhD
Professor, College of Pharmacy, Florida Agricultural and Mechanical University

Leonard Petrucelli, PhD, Assistant Chair
Chair, Department of Neuroscience and Professor of Neuroscience, Mayo Clinic Jacksonville

Neurologists:
Mark Brody, MD, CPI
President and Founder, Brain Matters Research

Neill Graff-Radford, MD
Professor of Neurology, Department of Neurology, Mayo Clinic Jacksonville

Hal S. Pineless, DO, FACN
President and Owner, NeuroCare Institute of Central Florida, PA

NATIONAL INSTITUTES OF HEALTH STATE RANKING IN TOTAL AMOUNT OF ALZHEIMER'S DISEASE RESEARCH FUNDING

Between fiscal years 2012-2014, Florida researchers were awarded $12,017,087 from the National Institutes of Health (NIH) to perform Alzheimer's disease research, ranking 12th, 13th , and 11th in national federal funding, respectively, per the NIH's National Center for Health Statistics. By fiscal year 2015, NIH funding in the state of Florida nearly doubled to $22,729,691 and nearly tripled in fiscal year 2017, to $60,454,514 (Figure 2). **Since the inception of the Ed and Ethel Moore Alzheimer's Disease Research Program in 2014, Florida has increased its national ranking to eighth place and its total federal funding for Alzheimer's disease research increased by $13,227,930 in the 2018 fiscal year** (Figure 1). Florida is one of two states in the southeastern United States to be ranked in the Top 10. This significant increase in federal research dollars can be attributed to the foundational support provided by the Ed and Ethel Moore Alzheimer's Disease Research Program for groundbreaking research and training. **Florida saw the 5th highest growth in new research funding, behind the states of Wisconsin, Ohio, Michigan, and Georgia** (Figure 2 and 3).[10]

Figure 1: National Institutes of Health Alzheimer's Disease Research State Funding and Rankings Fiscal Year 2018

State	Total Funding	Rank
CA	$362,862,489.00	1
NY	$179,608,938.00	2
MA	$161,754,940.00	3
PA	$111,528,133.00	4
MD	$81,405,679.00	5
TX	$81,222,812.00	6
IL	$76,093,586.00	7
FL	$73,682,444.00	8
MO	$70,752,429.00	9
NC	$61,251,957.00	10
MN	$44,456,658.00	11
WA	$39,943,551.00	12
WI	$35,251,768.00	13
OH	$30,446,550.00	14
IN	$29,845,541.00	15
MI	$29,809,022.00	16
GA	$29,523,372.00	17
KY	$28,325,046.00	18
AZ	$25,671,108.00	19
CT	$23,164,775.00	20

Fig.1 NIH Research Funding from the 2018 Fiscal Year Reporting Period: The top twenty ranked states in NIH funding for Alzheimer's disease are displayed. With over $73.7. million in NIH funding, Florida is ranked EIGHTH in the nation. *Source: National Center for Health Statistics, National Institutes of Health 2018*

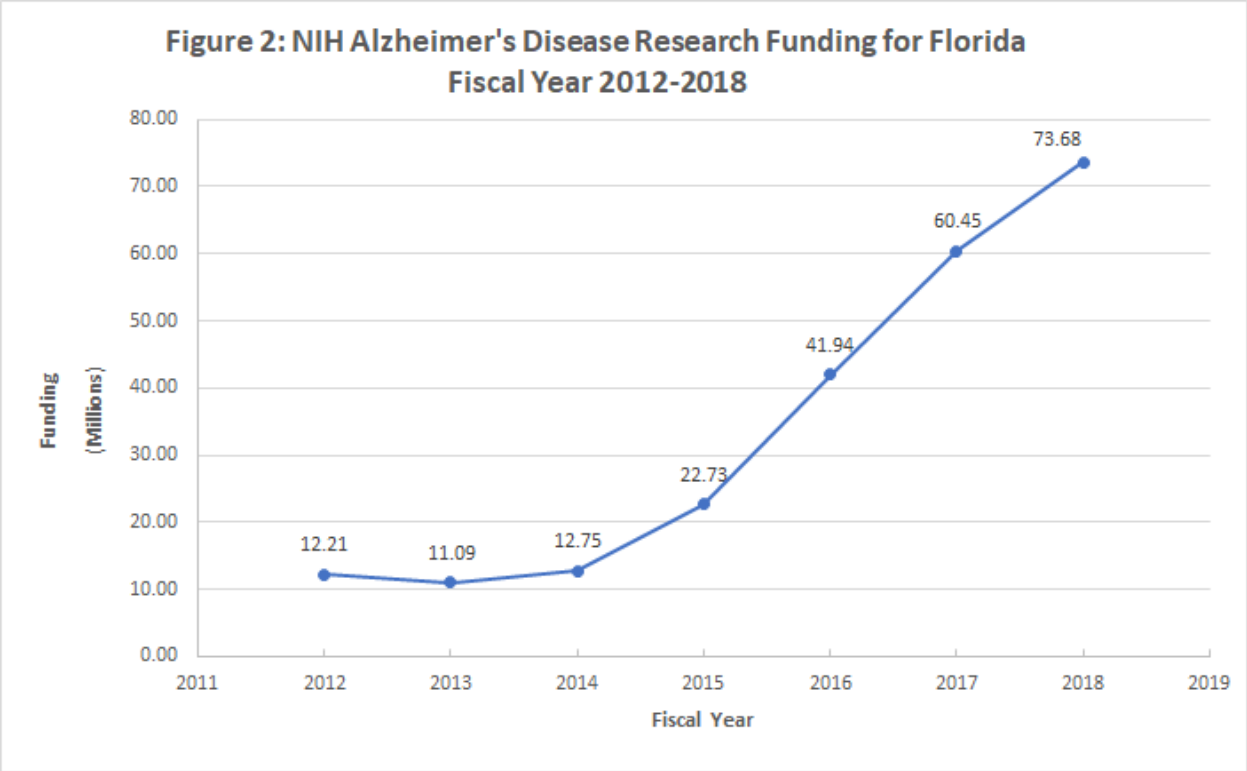

Fig. 2 NIH Research Funding Trends in Florida Fiscal Year 2012-2018: This chart illustrates the recent trends in federal funding for Alzheimer's disease research in the state of Florida. Following three years of relative stability in funding levels, fiscal years 2015-2018 saw a vast increase of funding leading to a 503% increase of funding since 2014. In fiscal year 2018, Florida's total federal funding increased by $13.23 million. *Source: National Center for Health Statistics, National Institutes of Health 2018.*

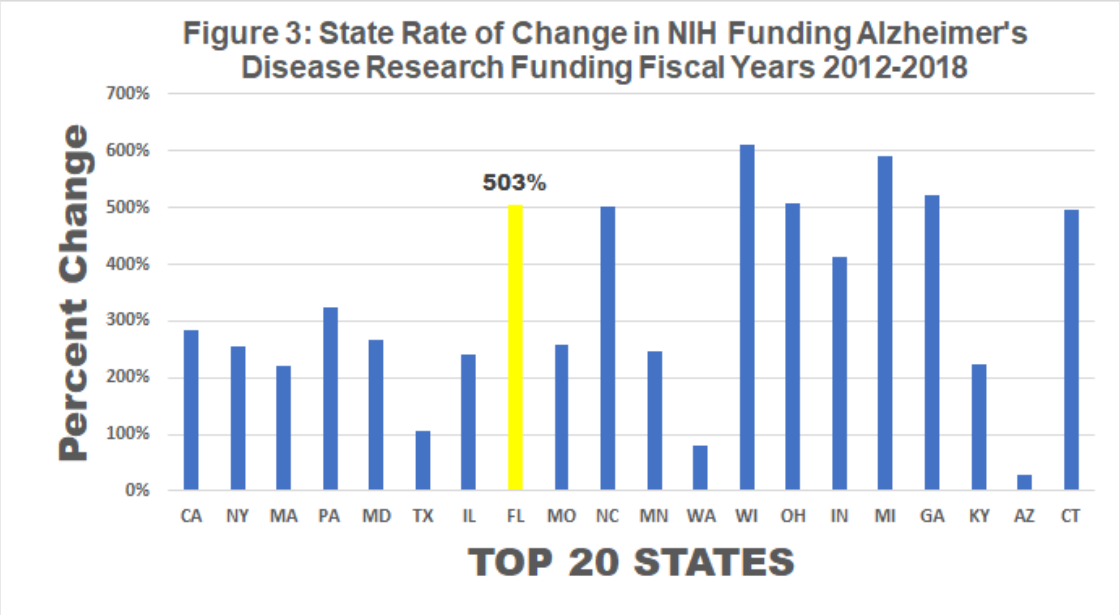

Fig. 3 Change in NIH Research Funding in the Top 20 States Fiscal Years 2012-2018: This graph displays the rate of change in federal Alzheimer's disease research funding for the Top 20 states for fiscal years 2012-2018. Among the Top 10 ranked states in NIH funding for Alzheimer's disease, Florida saw the fifth highest funding gains since 2012 (503%). Between 2017 and 2018, Florida's NIH funding for Alzheimer's disease grew by 21.9% and was ranked eight for total funding. *Source: National Center for Health Statistics, National Institutes of Health 2018.*

PROGRESS TOWARD PROGRAMMATIC GOALS

The Ed and Ethel Moore Alzheimer's Disease Research Grant Advisory Board's research agenda emphasizes the creation of intra-state research collaborations to make progress toward Florida becoming the premier state for Alzheimer's disease prevention, diagnosis, treatment, and ultimately, cure for this disease. The research agenda has five research priority areas that are outlined in the Funding Opportunity Announcement (FOA) and are listed below:

- The social/behavioral aspects of care, as well as palliative and end of life care for people with Alzheimer's disease
- Elucidation of the basic science relating to the disease
- Consortium grants between Florida-based institutions to augment established research networks and promote novel networks
- Epidemiological studies examining the prevalence, incidence, and risk factors of the disease with priority given to studies examining health disparities
- Fellowships aimed at enhancing the workforce of Florida's researchers working on Alzheimer's disease

This fiscal year, the legislature provided $5 million for research grants. Appendix A details all newly awarded grants and Appendix B details all active grants in 2019-2020. Information regarding progress reports, follow on funding, publications, and patents of each active grant is found in Appendix B.

RECOMMENDATIONS FROM THE ALZHEIMER'S DISEASE RESEARCH GRANT ADVISORY BOARD TO FURTHER THE MISSION OF THE PROGRAM

This has been a successful year for the Ed and Ethel Moore Alzheimer's Disease Research Grant Program with funding to award $5,000,000 among 22 outstanding research projects this fiscal year. Without this support, the eminent scientific advancements and discoveries in Alzheimer's disease would not be possible.

Statutory change is needed to allow for reimbursement of travel expenses resulting from Advisory Board in-person meetings. Face-to-face communication intensifies the exchange of information to allow for effective strategic planning and in-depth communication about critical research issues. In-person meetings engage full attention, build trust and credibility of the Advisory Board, and strengthen collaboration on ideas from their expertise that may be translated into research priorities for grant applications. These meetings can fuel more and varied planning to hone the Research Agenda and the Funding Opportunity Announcement documents to identify unaddressed needs and challenges of researchers as they work to discover or enhance preventions, care, cures, and treatments of Alzheimer's disease.

The Alzheimer's Disease Research Grant Advisory Board thanks the Governor and the Florida Legislature for their continuous support as we work together to eradicate Alzheimer's disease.

Appendix A
FISCAL YEAR 2019-2020 NEWLY AWARDED GRANTS (effective December 15, 2019)

Grant #	Organization	Principal Investigator	Project Title	Award Amount
20A01	University of South Florida	Woo, Jung A.	Divergent SSH1 Signaling in Mitochondria Toxicity and Clearance	$250,000
20A02	University of Miami Miller School of Medicine	Loewenstein, David	Postdoctoral Fellowship in Neuropsychology and Cognitive Neuroscience	$87,959
20A03	Florida Atlantic University	Ordonez, Maria	Opening and Sustaining Loving, Caring Conversations for Advance Care Planning for Patients and Families Living with Alzheimer's Disease	$100,000
20A04	University of Miami Miller School of Medicine	Curiel Cid, Rosie	Postdoctoral Fellowship in Neuropsychology	$86,211
20A05	Bascom Palmer Eye Institute/University of Miami	Jiang, Hong	Retinal Biomarkers for Monitoring Vascular Contributions to Alzheimer's Disease	$250,000
20A06	University of South Florida	Wang, Lianchun	The Role of Extracellular Tau in Endothelial Cell Biology	$250,000
20A07	Mayo Clinic Florida	McLean, Pamela	Modeling Lewy Body Dementias: Towards a Better Understanding of Amyloid-Beta and Alpha-Synuclein in ADRDs.	$99,000
20A08	University of Florida	Armstrong, Melissa	Communication of Dementia Diagnoses: Investigating Patient, Family, & Physician Experiences and Developing Best Practices	$374,660
20A09	Florida State University	Wilber, Aaron	Cortical-Hippocampal Interactions During Sleep in Alzheimer's Disease	$250,000

Grant #	Organization	Principal Investigator	Project Title	Award Amount
20A10	Mayo Clinic Jacksonville	Das, Pritam	Detection of Vascular and Inflammatory Plasma Biomarkers in Patients Diagnosed	$250,000
20A11	University of Miami Miller School of Medicine	Crocco, Elizabeth	Building an Advanced Cognitive and Biomarker Registry for African American Older Adults At-Risk for Alzheimer's Disease	$248,590
20A12	Florida Atlantic University	Barenholtz, Elan	Development of a Gaze and Speech-Behavior Based Cognitive Exam to Assist in the Detection of Early-Stage Alzheimer's Disease and Related Disorders	$99,863
20A13	Carlos Albizu University	Rodriguez, Miriam	Relationship Between Functional Measures, Cognitive Performance, and AD Biomarkers Between Hispanic and White Non-Hispanic Older Adults	$250,000
20A14	University of Miami	Wahlestedt, Claes	Contributions of Histone Deacetylase 8 (HDAC8) to Alzheimer's Disease Pathogenesis	$249,959
20A15	University of Florida	Ebner, Natalie	Determining Plasticity of Brain-regulatory Mechanisms Related to Emotion Processing: A Neurofeedback Approach in Older Adults with Amnestic Mild Cognitive Impairment	$249,930
20A16	University of Florida	Burke, Sara	Cyclic Ketogenic Therapy as Treatment for Alzheimer's Disease-Related Metabolic Decline, Tau Pathology and Cognitive Impairments	$250,000

Grant #	Organization	Principal Investigator	Project Title	Award Amount
20A17	The Brain Institute at Florida Atlantic University	Zhang, Qi	Amyloid Precursor Protein and Cholesterol as a Novel Druggable Axis for Alzheimer's Disease	$100,000
20A18	University of South Florida	Taheri, Saeid	Impact of Cerebrovascular Pathology on Alzheimer's Disease and Other Dementia	$250,000
20A19	University of Miami	Alperin, Noam	Lifestyle Stressors of Hippocampus and AD Related Brain Regions: Potential for Intervention	$249,999
20A20	Mayo Clinic Florida	Robinson, Maisha	Between Here and There: Addressing End-of-Life Disparities Among African Americans with Mild Cognitive Impairment and Dementia Through Community-Based Training in Advance Care Planning	$238,665
20A21	Mount Sinai Medical Center	Duara, Ranjan	Utility of Blood Biomarkers for Amyloid, Tau and Neurodegeneration to Assist in the Diagnosis of Alzheimer's Disease and Other Dementias – Relationship to Cognition, Brain Atrophy and Amyloid Load	$250,000
20A22	Mayo Clinic Jacksonville	Carter, Rickey	Racial and Ethnic Differences in Gene Expression Data	$250,000

NEW GRANTS FISCAL YEAR 2019-2020 (Effective December 15, 2019)
(Funding Year 2019-2020)

1. **Grant #20A01:** Divergent SSH1 Signaling in Mitochondria Toxicity and Clearance

   Principal Investigator: Jung A. Woo, Ph.D

   Organization: University of South Florida

   Abstract of Proposed Research: Accumulations of toxic protein assemblies (i.e. Abeta & tau) and dysfunctional mitochondria are associated with synaptic and neuronal loss in multiple neurodegenerative disorders, including Alzheimer's disease (AD). Such pathogenic accumulations are thought to arise in large part from clearance defects in the autophagy--lysosome system. While mounting experimental evidence supports the notion that AD is a tauopathy driven Abeta, there is still a considerable knowledge gap in understanding the role of mitochondrial dysfunction and clearance in AD pathogenesis. The recently published and preliminary studies indicate that the Slingshot homolog-1 (SSH1) pathway constitutes a critical link in mitochondrial dysfunction and clearance in the Abeta--tau neurotoxic signaling cascade.

   SSH1 is a protein enzyme classically known for its cofilin activating function by a process called 'dephosphorylation'. Activated cofilin can then sever F--actin at the synapse and/or translocate to mitochondria to promote mitochondria--mediated cell death. Likewise, researchers have found that activation of SSH1 and cofilin are required for Abeta oligomer--induced mitochondrial dysfunction, synaptic loss, as well as deficits in LTP and learning/memory in cellular and mouse models of Abeta pathogenesis. In support of these findings, activated cofilin and SSH1/cofilin complexes are increased in AD brains and AD mouse models.

   In preliminary studies, research staff found that the SSH1 protein contains 2 functionally divergent activities that impact mitochondrial homeostasis: 1) cofilin--mediated mitochondrial dysfunction; and 2) inhibition of clearance of dysfunctional mitochondria by mitophagy. While the first activity via cofilin is well known, the second activity is entirely novel. Staff have also found that these two activities are modular and fully separable. Researchers *hypothesize that both functional modalities of SSH1 contribute to mitochondrial homeostasis and synaptic toxicity in AD pathogenesis.* In this proposal, staff combine state-of-the--art approaches to dissect these 2 divergent functions of SSH1 on mitochondria in the hopes of providing molecular insights to mitigate AD pathogenesis.

2. **Grant #20A02:** Postdoctoral Fellowship in Neuropsychology and Cognitive Neuroscience

   Principal Investigator: David Loewenstein, PhD

Organization: University of Miami Miller School of Medicine

Abstract of Proposed Research: Clinical researchers across medicine need to acquire a set of core competencies and skills in order to effectively contribute to the ever--evolving field of Alzheimer's disease research. The early detection of AD is of critical importance as the population ages, and emerging clinician scientists are required to have knowledge about biology and cognition, while also developing clinical skills to understand risk, progression, and impact of intervention. Moreover, leadership, team science, study management, data management, and grant writing are only a few of the many pillars that must be developed and nurtured during the training years in order to prepare future investigators for an independent career in AD clinical research. This group of investigators at the Center for Cognitive Neuroscience and Aging (CNSA) have extensive expertise and track record in training the next generation of neuropsychologists, cognitive neuroscientists, and geriatric specialists. Graduates have developed advanced skills in clinical, cognitive and functional assessment, research methods, grant writing, test development, and cognitive remediation in a widely diverse platform of older adults at--risk for developing of neurodegenerative disorders. Central to this research has been the development of a state--of--the--art and multimodal suite of biological markers of AD pathology that serve as an excellent platform to train emerging investigators about genetics, peripheral inflammatory markers, cerebrospinal fluid collection and analyses to measure AD pathology, and various neuroimaging modalities including MRI, amyloid PET/CT, and tau PET/CT. This fellowship will especially emphasize the integration of biological markers in cognitive research and cognitive neuroscience.

The proposed one--year postdoctoral research fellowship will offer specialty training in Alzheimer's disease and related disorders to an individual with predoctoral training in neuropsychology and cognitive neuroscience. The diverse population that is served at the CNSA, the current research portfolio and the mentoring team is well-poised to provide a comprehensive training opportunity and prepare an emerging specialist for a career in AD clinical research.

The mentorship team has a longstanding history of training postdoctoral fellows. Dr. David Loewenstein, CNSA Director, has decades of experience in educating and preparing postdoctoral trainees and early stage investigators, and has been an active Ed and Ethel Moore Fellowship Mentor since the inception of the program. Dr. Loewenstein would serve as the Primary Mentor of this award given his expertise related to cognitive change and various biomarkers of AD brain pathology. Co--Mentor, Dr. Rosie Curiel Cid, has also mentored postdoctoral fellows for over eight years and would play an active role given her and Dr. Loewenstein's productive joint program of research at the CNSA, which includes three active longitudinal RO1 studies funded by the National Institute on Aging, and several active Ed and Ethel Moore AD research studies, that will serve as the training platform for the postdoctoral candidate.

3. **Grant #20A03:** Opening and Sustaining Loving, Caring Conversations for Advance Care Planning for Patients and Families Living with Alzheimer's Disease

   Principal Investigator: Maria Ordóñez, DNP, APRN

   Organization: Florida Atlantic University

   Abstract of Proposed Research: The Louis and Anne Green Memory and Wellness Center (MWC) of the Christine E. Lynn College of Nursing (CON) at Florida Atlantic University (FAU), the Charles E. Schmidt College of Medicine (COM) at FAU, and the FAU Office of Interprofessional Education (OIPE) created an interprofessional team (nursing, medicine, education, social work, others) to work on a research project in response to the Florida Department of Health Ed and Ethel Moore Alzheimer's Disease Research Program Funding Opportunity– Priority Area 1.3.1 Advance Care Planning (ACP). The project, titled *Opening and Sustaining Loving, Caring Conversations on Advance Care Planning for Patients and Families Living with Alzheimer's Disease,* focuses on conducting timely, meaningful conversations about ACP with persons living with Alzheimer's Disease (AD) and their family caregivers (CGs), and educating them on the importance and benefits of understanding and completing ACP. Researchers want to change and simplify current unclear and negative language used in ACP documents and processes to words and phrases that are easily understood and express comfort, love, and caring. Also, staff want to emphasize a gap in the current language as there are no statutory US state advance directives that specifically address AD, the sixth leading cause of death in US, as a condition to change level of care. The goal of the project is promotion and best practices for conducting ACP with persons living with AD and their CGs using all available tools, including online advance directives. The team will create a simple ACP toolkit to be shared with persons living with or at risk for AD and their CGs at the MWC and further promote ACP (discussions and completion) through primary care services and other community healthcare specialties by developing a brief train--the-trainer program.

   The MWC houses a state--designated, and partially funded Memory Disorder Clinic (MDC) under the Florida Department of Elder Affairs (DOEA) - Alzheimer's Disease Initiative and an Adult Day Center licensed by the Agency for Health Care Administration, the first in Florida to be designated as a Specialized Alzheimer's Service Center. The MWC is therefore positioned as an ideal practice site and pillar for the proposed project. The MWC provides an array of dementia specialized programs of care and supportive services for persons living with or at risk for AD or Related Dementias (ADRD) and their CGs in collaboration with the patients' existing healthcare team. Currently, 29.5% of new patients seen at the MWC have completed ACP, which aligns with the national averages reported by the Centers for Medicare and Medicaid Services and the National Institutes of Health. The MWC actively collaborates with a statewide network of 17 MDCs and participates with other DOEA activities, including the Dementia

Care and Cure Initiative and the Florida State Health Improvement Plan and its new Priority 9 with a specific focus on ADRD.

The interprofessional grant team has the clinical geriatric practice, educational, and research skills and expertise to successfully complete the proposed project and expand in the future. The Principal Investigator is the Director of the MWC, FAU MDC Coordinator, and Associate Professor in Practice-Teaching track at CON; Co-Investigators are the Director of COM Professional Education--Simulation Center, an Associate Professor of Hospice and Palliative Medicine at COM, and the Director of OIPE.

4. **Grant #20A04: Postdoctoral Fellowship in Neuropsychology**

Principal Investigator: Rosie Curiel Cid, PsyD

Organization: University of Miami Miller School of Medicine

Abstract of Proposed Research: The Center for Cognitive Neuroscience and Aging (CNSA) has a robust and growing program of state and federally funded research devoted to developing novel diagnostic assessment paradigms and tools to detect preclinical Alzheimer's disease (AD) and related disorders. Moreover, the CNSA is home to the state--funded University of Miami Memory Disorders Clinic (UM), and the clinician--scientists are active co-investigators on several AD--related projects including the 1Florida Alzheimer's Disease Research Center. This rich training environment has served as the platform upon which this organization has successfully and continuously trained Ed and Ethel Moore Postdoctoral Research Fellows since the program was initiated during the 2015--2016 year.

The focus of the research fellowship is to offer a promising postdoctoral candidate the opportunity to receive specialty training in Alzheimer's disease by: a) developing enhanced clinical evaluation and diagnostic skills, b) participate in ongoing clinical research projects that are studying promising new methodologies to improve the clinical assessment of diverse older adults at risk for the development of AD and related disorders, c) learn about cross--cultural neuropsychological assessment and the development of culturally fair diagnostic assessment instruments, which is of critical relevance in the State of Florida and, d) receive training in writing federally funded grant applications to prepare him/her to become an independent investigator.

Competent clinical assessment that is sensitive enough to detect preclinical AD remains a critical priority area in Alzheimer's disease research. Offering this training opportunity to a neuropsychologist is of particularly high impact, as this discipline plays a direct and critical role in Alzheimer's disease clinical research. In addition, the longitudinal nature of the research program will offer the unique opportunity for the fellow to assist with longitudinal data analysis, expose them to state--of--the--art cognitive assessment

methods and various biological markers of AD pathology including amyloid and tau neuroimaging and cerebrospinal fluid markers.

Drs. Rosie Curiel Cid and David Loewenstein would serve as primary and secondary mentors, respectively. Together, they have three active longitudinal RO1 studies (Loewenstein--PI and Curiel-PI) funded by the National Institute on Aging, three active Ed and Ethel Moore AD research studies, and are active members of the state--funded University of Miami Memory Disorders Clinic. This focused and highly productive program of research at the CNSA, along with the mentors' longstanding background in training post-doctoral fellows and junior faculty, offers an unparalleled specialty training opportunity for the postdoctoral candidate to expand their competency to serve diverse older adults who are at risk for the development of neurodegenerative conditions such as AD.

5. **Grant #20A05:** Retinal Biomarkers for Monitoring Vascular Contributions to Alzheimer's Disease

   Principal Investigator: Hong Jiang, MD, PhD

   Organization: Bascom Palmer Eye Institute /University of Miami

   Abstract of Proposed Research: Accumulating evidence implicates the significance of the vascular contributions to Alzheimer's disease (AD). Cerebral small vessel disease, especially at the smallest vessel (i.e. capillary) level, is regarded as a major pathogenic contributor to dementia. Targeting the vascular component may help to potentially slow down the disease progression. Currently, there are no easily accessible and reliable ways to screen for vascular contributions to cognitive impairment and dementia (VCID). The ability to visualize and assess the cerebral microvasculature *in vivo* would certainly address that issue, but current technologies are difficult in practice, and not practical for population-based screening. The retina is an extension of the brain and shares similar anatomic and physiologic vascular features with the brain. The transparent ocular media allows in vivo visualization and access to retinal microvasculature and offers an innovative solution to the limitations encountered with imaging of the brain. The retina has been used as a model to study disorders of the central nervous system (e.g., stroke and AD). The alterations of retinal microstructure and microvasculature have been suggested to reflect similar changes occurring in the brain. For example, the thinning of the retinal nerve fiber layer and ganglion cell layer is regarded as a potential image marker of cerebral neurodegeneration in AD. Previous studies have identified a spectrum of retinal vascular alterations visible in fundus photos in patients with cognitive impairment. The preliminary study found that the alterations in the retinal capillary network and retinal tissue perfusion differentiated older adults with healthy cognition from patients with AD and mild cognitive impairment (MCI). Researchers hypothesize that retinal microvascular alterations are related to cognitive impairment and can be used as the potential markers of VCID. Staff propose to use existing patient cohorts in the current NIH R01 studies (PIs: Loewenstein R01 AG047649 and Curiel R01

AG055638-01A1) to examine retinal microvascular changes at the capillary level in relation to cognitive impairment in patients. Using optical coherence tomography angiography and a highly novel form of fractal analysis of retinal microvasculature, staff will determine volumetric vessel density, indicating tissue perfusion. In addition, staff will determine the most profound alteration of retinal neuronal layers (retinal nerve fiber layer and ganglion cell-inner plexiform layer). The retinal variables will be correlated to white matter hyperintense volume, total brain volume, Amyloid load, and cognitive function. The effect modification of vascular risk factors, medications, and lifestyle will be adjusted for determining the relationship of retinal vascular alteration and cognitive impairment. The proposed study will not overlap with these funded RO1 studies. However, the valuable database, including patient cohorts, amyloid scans, MRI data, and cognitive function test results, will be used for analyses. The goal is to develop and optimize retinal microvascular alterations into tangible markers which could be readily used in research and clinical trials for risk stratification, treatment refinement, and outcome evaluation.

6. **Grant #20A06:** The Role of Extracellular Tau in Endothelial Cell Biology

   Principal Investigator: Lianchun Wang, MD

   Organization: University of South Florida

   Abstract of Proposed Research: The importance of vascular contributions to cognitive impairment and dementia (VCID) associated with Alzheimer's disease (AD) and related neurodegenerative diseases is increasingly recognized, however, the underlying mechanisms remain obscure. A very recent study reported that aged tau-overexpressing, not the amyloid b overproduction in mice develop changes to blood vessels including abnormal, spiraling morphologies, but its underlying cellular and molecular mechanisms remain largely unknown. The preliminary study uncovered that tau exerts a direct angiogenic effect on brain endothelial cells (BEC) including tau internalization. Based on preliminary study findings and current literature, staff have hypothesized that extracellular tau disturbs BEC functions attributing to tauopathy/AD. Staff will test this novel hypothesis using recombinant full--length tau, *in vitro* BEC function assays and an *in vivo* angiogenesis model. The proposed new studies are anticipated to establish a novel concept: Tau is a novel angiogenetic factor but functions to disturb BEC function and brain blood vessel integrity, revealing a molecular and cellular mechanism understanding of tauopathy from angiogenesis aspect.

7. **Grant #20A07:** Modeling Lewy Body Dementias: Towards a better understanding of Amyloid-Beta and Alpha-Synuclein in ADRDs.

   Principal Investigator: Pamela McLean, PhD

   Organization: Mayo Clinic Florida

Abstract of Proposed Research: Modeling Lewy body dementias: Towards a better understanding of amyloid--beta and alpha-synuclein in ADRDs. After Alzheimer's disease (AD), Lewy body dementia (LBD) is the most common form of dementia and is classified as one of the major Alzheimer disease and related dementias (ADRDs). There is extensive clinical overlap between AD and LBD with LBD patients often facing diagnostic uncertainty that leads doctors to prescribe AD medications that can significantly worsen LBD symptoms. LBD falls in the category of neurodegenerative disorders with pervasive comorbid neuropathology; in this case amyloid--beta (Aβ) and alpha--synuclein (α-syn) neuropathologies are key aspects of the disease. To accurately diagnose LBD, effectively treat it, halt its progression, and develop a cure, requires an understanding of the molecular mechanisms and disease progression. Despite their limitations, rodent models continue to play a key role in advancing understanding of dementias and related neurodegenerative disorders and are valuable research tools to decipher mechanisms of disease. The major goal of this pilot grant application is to develop a rodent model of LBD that recapitulates the key symptoms, neuropathologies, and progression of the disorder, facilitating investigations into the contribution of Aβ and α-syn to disease pathogenesis, and creating a useful preclinical model for biomarker and therapeutic agent discovery. Herein staff propose to dissect in vivo Aβ/α-syn crosstalk to elucidate how Aβ and α-syn exert their neurodegenerative effects using a novel mouse model with temporally controlled Aβ and α-syn co--pathology. Researchers will use intravenous adeno-associated virus (AAV) technology to transduce developmentally mature neurons in the brains of AD transgenic mice with α-syn before, during, or after the development of Aβ pathology. Overall, the objective of this pilot proposal is to establish a preclinically useful rodent model of LBD and identify how Aβ and α-syn interplay results in neuronal dysfunction and neurodegeneration. Behavioral, histological, and biochemical analyses will be conducted to generate insight into Aβ/α-syn interactions in LBD.

8. **Grant #20A08:** Communication of Dementia Diagnoses: Investigating Patient, Family, & Physician Experiences and Developing Best Practices

   Principal Investigator: Melissa Armstrong, Md, MSc

   Organization: University of Florida

   Abstract of Proposed Research: Dementia is a brain disease that causes a decline in a person's memory and thinking that affects everyday life. Dementia can be due to various medical problems, including Alzheimer's disease, Lewy body disease, and stroke. Current research suggests that people living with memory and thinking problems and their families want to know what's wrong and receive a diagnosis. Doctors, though, may not want to give a specific diagnosis like Alzheimer's disease. This can be because the doctor doesn't feel confident in the diagnosis, the diagnosis is difficult, or the doctor is not sure that the diagnosis will change treatment. There are no current standards outlining when doctors should give dementia diagnoses to people with memory and

thinking concerns and their families. There are also no standards about how this information should be given. In this study, research staff will interview individuals with memory and thinking problems, family members, and doctors about their experiences in receiving and giving dementia diagnoses. Staff will learn what went well and what went badly. Staff will ask what made the experience better or worse, and what challenges there were to receiving or giving the diagnosis. Staff will ask individuals with memory and thinking problems and their family members what they want to know about a diagnosis and how they want to hear that information. Staff will then analyze the information from these interviews to identify how people's experiences and views were similar or different. After the interviews, staff will gather a group of people with memory and thinking problems, family members, and doctors. Staff will review the results of the interviews and what prior research tells us about giving and receiving a diagnosis of dementia. Then as a group staff will develop proposed best practice standards for how doctors should give a diagnosis of dementia to patients and families. The proposed best practice standards will take into account the views of people with memory/thinking problems, families, and doctors. The standards will address the fact that people's wishes about hearing the diagnosis may be different. At the end of the study, researchers hope to have information and standards that can help doctors better give the diagnosis of dementia to people who want to receive it.

9. **Grant #20A09:** Cortical-Hippocampus Interactions During Sleep in Alzheimer's Disease

   Principal Investigator: Aaron Wilber, PhD

   Organization: Florida State University

   Abstract of Proposed Research: Alzheimer's disease is devastating for individuals and society. Impaired learning and memory, particularly in the context of spatial navigation, is one of its major symptoms. Similarly, rodent models of Alzheimer's disease also exhibit impairments in spatial navigation. A preponderance of evidence suggests abnormal cortical--hippocampal communication, including between the parietal cortex (PC) and hippocampus in humans with Alzheimer's disease. Memory reactivation is critical to consolidation of newly acquired memories. However, no studies have assessed changes in memory reactivation in rodent models of Alzheimer's disease. Abnormal reactivation could cause impaired learning in Alzheimer's disease, especially in early (pre--symptomatic) stages of disease progression as researchers propose here. To develop a model for assessing potential contributions of altered cortico--hippocampal function to Alzheimer's disease, the proposed research will explore the functionality of memory reactivation in the hippocampal--PC network in animal models of Alzheimer's disease. To do this, staff will use a triple transgenic mouse model of Alzheimer's disease where three major genes associated with familial Alzheimer's disease are expressed. This mouse model mimics plaque and tangle pathological hallmarks of the disease with a distribution pattern similar to human patients. In addition, all findings will be confirmed in a new mouse model in which mouse amyloid beta (Aβ) sequence is replaced with non-mutated human Aβ sequence to more closely mimic sporadic Alzheimer's in

humans (hAβ-KI). Specifically, staff will: 1) assess rest--related memory replay within and across the hippocampus and PC; 2) use a novel targeted optogenetic approach to functionally dissect the relative contributions of Tau and Aβ in the hippocampus and PC to impaired memory reactivation. This project will provide insight into the normal function of a circuit that is dysfunctional in Alzheimer's disease and allow us to probe dysfunction in this circuit that emerges in very early stages of disease progression in mouse models of Alzheimer's disease, so that staff can begin to understand changes in this network which may underlie the emergence of cognitive impairments observed in Alzheimer's disease. The focus of this research is on the functional circuit mechanisms affected by amyloid beta deposition in Alzheimer's disease. This research is relevant to public health because it will increase knowledge about the role of changes in the functional interactions between the cortex and hippocampus and use current technologies to perform a functional dissection of amyloid beta clearance in these critical brain circuits. The proposal will expand the knowledge base and establish a new research platform for understanding the mechanism of impaired memory in Alzheimer's disease.

10. **Grant #20A10:** Detection of Vascular and Inflammatory Plasma Biomarkers in Patients Diagnosed

    Principal Investigator: Pritam Das, PhD

    Organization: Mayo Clinic Jacksonville

    Abstract of Proposed Research: The long--term objectives of this proposal are to identify novel biomarkers of disease and to reveal mechanistic insight in cerebral small vessel disease (CSVD) and vascular cognitive impairment and dementia (VCID), the second most common cause of dementia. CSVD is a highly prevalent condition among older adults, with many stigmata readily seen on brain MRI, including white matter hyperintensities (WMH), microbleeds, lacunar infarcts, and enlarged perivascular spaces. Despite advances in the fields of neuroimaging and genomics, the molecular pathogenesis of CSVD is still poorly understood, with no drug specifically approved to reverse, halt, or even forestall progression. Early cerebrovascular dysfunction and blood--brain barrier (BBB) damage have been proposed as initiators of sporadic CSVD. This application takes advantage of a human experiment of nature in hypoxia and oxidative stress, namely obstructive sleep apnea (OSA). A meta--analysis of 22 studies found that OSA doubled the prevalence of WMH and that patients with WMH had significantly higher apnea--hypopnea indices (AHI). Recently, the group found that cultured brain endothelial cells (ECs) under hypoxic conditions showed aberrant vascular endothelial growth factor (VEGF) receptor expression patterns and increased expression of inflammatory markers, leading to endothelial cell senescence. Suppression of specific VEGF receptor expression prevented the up-regulation of inflammatory markers in hypoxic ECs, suggesting a direct role of altered VEGF receptor expression and signaling events in this paradigm. Furthermore, staff have generated preliminary data showing that patients diagnosed with OSA with severe white matter pathology, have increased levels of circulating plasma CXCL10 levels and neurofilament light chain, a specific

marker for neurodegeneration. Researchers hypothesize the hypoxic conditions in the brain leads to dysregulation of individual VEGF receptors in ECs, leading to cerebrovascular dysfunction and ultimately CSVD. The scientific premise of this proposal is to further investigate aberrant VEGF receptor signaling events in the hypoxic brain and utilize this knowledge to identify novel biomarkers early in the disease process in OSA and SCVD. The specific Aims are as follows: *Aim 1.* Measure changes in vascular, inflammation biomarkers and neurofilament light chain in the plasma of patients diagnosed with OSA with varying degrees of CSVD pathology using the Mayo Clinic Familial Cerebrovascular Diseases Registry (IRB: 08-003878). *Aim 2.* Perform a pilot prospective longitudinal study to measure changes in plasma biomarkers, cognitive impairment and white matter pathologies in OSA patients enrolled in the Mayo Clinic sleep center. *Aim 3.* Validate altered expression patterns of VEGF receptors and inflammatory/senescence markers in cerebral micro-vessels in autopsied human brain samples with pathologically--confirmed CSVD and vascular dementia. The proposed studies will provide a better understanding of the roles of individual VEGF receptor signaling events and their potential contribution to EC dysfunction and cerebrovascular damage in oxidative stress from sleep apnea. This could lead to biomarker development and drug targets for prophylaxing against CSVD in patients with cardiovascular risk and high oxidative stress.

11. **Grant #20A11:** Building an Advanced Cognitive and Biomarker Registry for African American Older Adults At-Risk for Alzheimer's Disease

    Principal Investigator: Elizabeth Crocco, MD

    Organization: University of Miami Miller School of Medicine

    Abstract of Proposed Research: The state of Florida's high ethnic and racial diversity provides a unique platform to address an array of scientific and clinical questions to refine current understanding of how Alzheimer's disease (AD) and Alzheimer's disease related disorders (ADRD) presents and progresses among the nation's diverse cultural groups. A growing body of evidence suggests that the prevalence of AD may be two-to--three times higher among older African Americans (AA) as compared to non--Hispanic whites. AAs are significantly under-represented in AD research and are generally less likely to utilize geriatric services. Some of the reasons noted for this disparity may include economic barriers, institutional racism, and mistrust of the medical system. Moreover, AAs are more likely to view significant memory loss in older age as an expected part of the aging process as opposed to a sign of disease.

    The project will leverage the expertise of investigators who have been actively and successfully working to engage AA communities in their research and clinical care. The aim of this project will be to build an advanced registry of 120 AA older adults at-risk for AD+ADRD using state-of-the-art and culturally-relevant novel cognitive tests, as well as advanced neuroimaging of the brain (i.e.: fMRI, structural MRI, amyloid PET/CT) and genetic analyses. This comprehensive work-up will: a) provide excellent and much

needed normative data for AA older adults, b) examine the relationships between genetic and epigenetic risk factors and brain imaging as it relates to cognition, and c) provide a critical pool of prospective participants for current federally funded projects including a large ongoing NIH-funded Alzheimer's Disease Research Center (ADRC), which is a close collaboration between the University of Miami, Mt. Sinai Medical Center, and the University of Florida.

Research staff will utilize strategies that have been previously shown to be efficacious in improving outreach efforts in AA communities. This includes building new and existing engagements with community stakeholders in an academic--community partnership to provide opportunities to foster education, ongoing dialogue, and a bridge that will establish the importance and value of research and its potential benefits to underrepresented minorities.

This project fills a critical gap and is expected to become the foundation of current and future NIH-funded projects in Florida that include this underserved group of individuals.

12. **Grant #20A12:** Development of a Gaze and Speech-Behavior Based Cognitive Exam to Assist in the Detection of Early-Stage Alzheimer's Disease and Related Disorders

    Principal Investigator: Elan Barenholtz, PhD

    Organization: Florida Atlantic University

    Abstract of Proposed Research: Current methods for diagnosing Alzheimer's disease and related disorders (ADRD) are often time-consuming, expensive, invasive, and inaccurate, with prodromal stage detection even more difficult and imprecise. Affected individuals are often not referred for testing until severe symptoms are noticed by the patient or their caregivers which can have consequences ranging from minor accidents to decreased health maintenance to life--threatening automobile crashes. Here, research staff propose to develop and test a new type of cognitive- behavioral exam that is fast, non-invasive, and automated. The approach uses machine-learning techniques applied to detailed eye movement and speech behavior data obtained during both directed and non-directed tasks. Staff propose that this method may allow for greater both directed and non-directed tasks. Staff propose that this method may allow for greater sensitivity/earlier diagnosis than pen-and-paper exams because it utilizes high - resolution, temporally-structured behaviors that are better able to reflect pathologies of the central nervous system. It may also serve as a diagnostic tool for those with reduced mobility. Finally, it allows for complete automation of both administration and analysis of the collected data, enabling easier detection of impairment by non--specialist physicians.

    Behavioral analytics, such as eye--movement tracking and speech analysis of verbal responses, are currently being used by several medical organizations for the detection of mental and neurological disorders including ADHD diagnosis and concussion detection. The scientific literature reports that several types of these behavioral measures have

shown some degree of efficacy in detecting ADRD and its pathology. These measures include smooth pursuit and simple saccades obtained from videonystagmography testing, reading saccades obtained from the King--Devick concussion test, and novelty--directed saccades in the visual paired comparison test. To date, no research (scientific or clinical) has been conducted that combines more than one type of behavioral measure into a single model for disorder classification, due in part to limitations in processing power and methodology. Staff propose to apply recently developed machine learning techniques for cognitive domain combination. The technique uses deep neural networks to analyze these and other identified behavioral measures in order to develop a diagnostic model for ADRD. The likely outcome of this research would be a diagnostic tool that would provide physicians with a probability score for diagnosis of ADRD, a measurement of severity (i.e. stage), and/or normalized scores for affected cognitive domains, e.g. explicit and implicit memory, attention, executive functioning, visuospatial processing, comprehension, and language production. The tool developed from this research could be deployed quickly and easily, without specialized training, in many clinical settings and may result in greater rates of detection of ADRD, potentially at an earlier stage of the disease.

13. **Grant #20A13:** Relationship Between Functional Measures, Cognitive Performance, and AD Biomarkers Between Hispanic and White Non-Hispanic Older Adults

    Principal Investigator: Miriam Rodriguez, PhD

    Organization: Carlos Albizu University

    Abstract of Proposed Research: The primary objective of this standard grant application is to compare functional measures that can effectively identify Alzheimer's disease (AD) risk factors at the earliest stages of the disease among Hispanic and white non--Hispanic participants in the 1Florida Alzheimer's Disease Research Center (ADRC). The 1Florida ADRC, an NIH-funded center, and Carlos Albizu University (CAU) have collaborated for several years, especially with respect to cross--cultural studies, including cognitive measures and AD biomarkers among Hispanic and non--Hispanic participants. Volumetric changes in the entorhinal cortex (ERC) and hippocampus (HPC) are noted in patients with mild cognitive impairment (MCI) and AD. Amyloid pathology becomes increasingly prevalent with age, but little is known about its effects on cognitive performance. The Apolipoprotein E (APOE) gene is recognized as a major factor in developing late onset AD. Given the genetic variability and the attenuated effect among Hispanics, it is important to compare the genetic contribution of ApoE4 status to AD pathophysiology among Hispanics and non-Hispanics. Previous studies conducted by the 1Florida ADRC have found that among Hispanic samples, amyloid load is strongly related to cognitive functioning in cognitively normal and early MCI stages, whereas regional atrophy on MRI scans has been found to be related to cognition in the late MCI and dementia stages.
    Parallel to the accumulation of AD biomarkers, functional decline takes place, and changes in functional status can be subtle and difficult to ascertain. Difficulties with

instrumental activities have been noted in patients with MCI. Since greater functional impairment has been associated with higher rates of progression to dementia, identifying the degree of functional impairment early in the course of the disease can have immediate implications for prognosis. Commonly used functional instruments include the Clinical Dementia Rating Scale (CDR) and the Alzheimer's disease Cooperative Study scale for ADL in MCI (ADCS-MCI-ADL). Few studies have examined relationships between AD biomarkers and functional measures among various ethnic groups.

With this application, research staff propose to examine the following functional measures: ADCS-MCI ADL (Galasko, 1998) ; the CDR (Morris, 1993), and staff will be introducing an additional functional scale, the modified Clinical Dementia Rating scale (mCDR; Duara et al, 2010). The latter uses a multiple-choice response format across the same six functional domains from the CDR, but unlike the CDR, the mCDR does not include any objective memory test. Preliminary comparisons between the CDR and mCDR by the 1Florida ADRC indicated the mCDR better predicted the transition from cognitively normal (CN) to MCI, whereas the CDR better predicted transition from MCI to AD. The relationship of these measures to cognitive performance, MRI volumetric analysis, Amyloid imaging, and Apoe4 carrier status will be examined among Hispanic and white non--Hispanic participants. Results of the proposed study will be an important contribution to understanding the effect of biomarkers, cognition, and ADL and IADL function among multi-ethnic groups. The study will help identify effective functional measures that can be used among Hispanics to diagnose and evaluate functional progression in AD and other dementias, especially in the prodromal stages when treatment can be most effective.

14. **Grant #20A14:** Contributions of Histone Deacetylase 8 (HDAC8) to Alzheimer's Disease Pathogenesis

    Principal Investigator: Claes Wahlestedt, MD, PhD

    Organization: University of Miami

    Abstract of Proposed Research: In the state of Florida alone, approximately half a million people currently suffer from Alzheimer's disease (AD). Considering the recent shortcomings of AD clinical trials, research staff and others have proposed targeting epigenetic enzymes as a potential therapeutic approach for AD. One such group of enzymes is Class I histone deacetylases (HDACs) comprising of 4 members: HDACs 1, 2, 3, and 8. Staff and others have shown that inhibition of HDACs 1, 2, and 3 can individually present beneficial effects for AD by either increasing neuroprotective genes such as Brain-Derived Neurotropic Factor (BDNF), reducing AD--like pathogenesis and/or increasing learning and memory in animal models. Remarkably little has been shown about the effects of HDAC8 activity on AD--like pathogenesis, although inhibition of HDAC8 has been well--documented as a drug target for the treatment of various types of cancers. Using validated tools from the HDAC8 cancer field, such as shRNAs and the selective HDAC8 inhibitor PCI 34051, preliminary data unexpectedly suggest that

interrupting HDAC8 signaling potentially aggravates AD--like pathogenesis. Indeed, following silencing of HDAC8 in an AD cell model, staff observed significant increases in expression of AD--related genes such as beta--secretases and gamma--secretase complex components, while noting decreases in BDNF mRNA levels. At the protein level, staff also found decreased BDNF, increased tau phosphorylation, increased Abeta1-42 and increased sAPP-beta (a cleavage product of beta--secretase) all contributors to AD hallmarks in patients' brain. Conversely, when staff increase HDAC8 expression in the brains of wild-type mice, staff observe cognitive enhancement in both the object recognition and object location memory tests. Researchers also observe increases in BDNF and phosphorylated cAMP element binding protein (CREB), both key components of learning and memory formation. These data support the hypothesis that HDAC8 activity is neuroprotective and potentially beneficial for Alzheimer's disease. Staff thus hypothesize that an HDAC8 activator will be protective against AD and result in increased cognitive performance in AD animal models. Through a screen of in-house libraries, staff have recently discovered a novel HDAC8 activator, CTI-701, that has favorable pharmacokinetic parameters and is more efficacious than the only commercially available HDAC8 activator, TM-2-51.

Here, staff plan to validate whether increased HDAC8 activity can prevent and/or rescue AD--like pathogenesis in the AD models. Staff will use both genetic (virus--mediated) and pharmacological (CTI- 701) interventions to increase HDAC8 activity. These studies will help confirm whether HDAC8 upregulation is a suitable target for AD and whether inhibition of this HDAC isoform should be avoided. The group has successfully generated and tested novel molecules that target epigenetic pathways in the context of AD in the past and anticipate obtaining positive effects with this HDAC8 activator.

Innovation and Significance: To date, this is the first group to show that HDAC8 inhibition exacerbates AD--like pathology. Data from the proposed work has the potential to validate whether increasing HDAC8 activity is protective, is a novel therapeutic strategy for AD, and yield a novel small molecule activator of HDAC8 suitable for AD treatment.

15. **Grant #20A15:** Determining Plasticity of Brain-Regulatory Mechanisms Related to Emotion Processing: A Neurofeedback Approach in Older Adults with Amnestic Mild Cognitive Impairment

    Principal Investigator: Natalie Ebner, PhD

    Organization: University of Florida

    Abstract of Proposed Research: In addition to cognitive changes, aging is associated with emotional changes, such as loss of interest in activities and lowered responding to emotional cues. These emotional changes occur in normal aging and tend to be pronounced (e.g., affective blunting, general apathy) in age--related neurodegenerative disorders such as Alzheimer's disease (AD). In addition to psychosocial factors (e.g., loss of spouse), there is evidence that age--related dampening of neural responses in

limbic regions crucially involved in emotion processing (i.e., anterior insula) may underlie emotional deficits in aging, with evidence of significant brain atrophy in the anterior insula in AD. Anterior insula supports the integration of internal signals from within the body with external signals in the environment and helps to simulate own and others' emotional states, contributing to successful social interaction and well-being. To date, processes in the brain underlying emotional deficits and the extent to which they are malleable in older adults and in age--related pathology are not well studied. Emotional health is relevant to people across all ages. Thus, it is vital to understand the extent to which emotions change as individuals grow older and to determine processes involved in effective regulation on the level of brain and behavior. To fill this research gap, researchers apply the innovative technology of real--time functional magnetic resonance imaging (rtfMRI), which the group has implemented at University of Florida for use in older adults, to directly test brain--behavior relationships. The specific aims are two-fold: 1) to examine whether patients with amnestic Mild Cognitive Impairment (aMCI) can learn to regulate brain activity in anterior insula, and 2) to test whether increasing anterior insula activity improves response to emotional cues. Staff will assess patients' anterior insula activity in real time and give them continuous feedback about how active it is. Staff will train patients to increase or decrease activity in anterior insula (or auditory cortex as a control) when asked to do so. Pre- and post--training, patients will assess images of people for emotional content and attempt to regulate their emotional responses to images of scenes and objects. The clinical group of aMCI patients potentially comprises an intermediate clinical stage between normal aging and AD dementia, one of the costliest public health concerns in the United States, with prevalence rates expected to triple in the coming decades. Studying this group provides an optimal timeframe for protective interventions targeting the neural mechanisms of pathology--related emotional deficits. Knowledge gained from this project will advance scientific understanding of the basic mechanistic chain underlying emotion processing in healthy and pathological aging. In addition, this project will implement rtfMRI as a novel neuroimaging technique for the study of brain--behavior connections in clinical older populations.

16. **Grant #20A16:** Cyclic Ketogenic Therapy as Treatment for Alzheimer's Disease-related Metabolic decline, Tau Pathology and Cognitive Impairments

    Principal Investigator: Sara Burke, PhD

    Organization: University of Florida

    Abstract of Proposed Research: A defining feature of Alzheimer's disease is a reduced ability of the brain to use glucose to meet its energetic needs. Relatedly, there is a well--established link between insulin resistance and the risk of developing Alzheimer's disease. Within the brain, dysfunctional insulin signaling can promote the pathological aggregation of tau protein that ultimately leads to neurodegeneration, brain atrophy, and cognitive loss. The pilot data, and the work of others, shows that high fat/low carbohydrate ketogenic diets have enormous potential for improving brain

metabolism and enhancing cognitive function. Ketogenic diets increase circulating and brain levels of ketone bodies that can substitute for glucose in the production of energy. While glucose utilization in the brain is impaired in Alzheimer's disease, the utilization of ketone bodies remains intact. Thus, ketogenic diet therapy is likely to improve brain metabolism in individuals with Alzheimer's disease thereby changing the disease course and enhancing cognitive function. *A major barrier to the implementation of nutritional ketogenic therapy, however, is low compliance associated with long--term carbohydrate restriction in Alzheimer's disease patients that have increased cravings for high carbohydrate, sweet foods.* The objective of this proposal is to restore brain metabolism in a rat model of age--related metabolic syndrome and Alzheimer's disease--related tauopathy through the use of a cyclic ketogenic diet. Cyclic ketosis allows for windows in which one can consume carbohydrates thereby showing greater translational potential than a standard continuous ketogenic diet. Pilot data in rats show that once an animal has established stable levels of ketosis over four weeks of a high fat/low carbohydrate diet, they can readily switch back into ketosis within 24 hours following a week of consuming a standard carbohydrate diet. A rat model will allow for precise control of caloric and micronutrient intake, which is difficult in community-dwelling populations, as well as sophisticated cognitive testing with touchscreen tasks that have been adapted from neuropsychological assessment in humans for use in rodents. Based on preliminary data, researchers will test *the central hypothesis* that after establishing stable ketosis for four weeks, 12 weeks of cycling on and off a ketogenic diet with 1 week intervals will be sufficient to normalize brain metabolism in rats with metabolic syndrome and tau pathology with the following aims: 1) *Determine if a cyclic ketogenic diet regimen can mimic long-term nutritional ketosis, and 2) Determine if cyclic ketosis can improve cognitive function in rats with tau pathology.* This work is significant because the successful completion of these aims will establish a novel therapeutic strategy with greater feasibility and translational potential than standard ketogenic diet therapies. Critically, ketogenic diet cycling is an accessible lifestyle modification that can be rapidly implemented in persons with Alzheimer's disease and at--risk populations.

17. **Grant #20A17:** Amyloid Precursor Protein and Cholesterol as a Novel Druggable Axis for Alzheimer's Disease

    Principal Investigator: Qi Zhang, PhD

    Organization: The Brain Institute at Florida Atlantic University

    Abstract of Proposed Research: Alzheimer's disease (AD), the predominant form of dementia, exhibits broad neuronal dysfunction in the early stage and progresses to significant neurodegeneration in the later stage. In addition to the well--known amyloid hypothesis, emerging evidence points to cholesterol as another key player in AD pathology. Major discoveries supporting this notion are: (1) cholesterol metabolism is autonomously regulated in the brain and found to be abnormal in the AD brain; (2) proteomic and lipidomic studies revealed coordinated decrease of neuronal membrane cholesterol (mChol) and synaptic proteins essential for neuronal function; (3) genetic

studies consistently identified ApoE4, an isoform of ApoE weak in transporting cholesterol, as the highest genetic risk factor for the most common sporadic AD; and (4) genes like ABCA7 and CYP46A1 that involve in cholesterol metabolism have also been repeatedly identified as AD risk factors.

Structural biologist Dr. Charles Sanders at Vanderbilt University, has identified a cholesterol--binding motif in the amyloid precursor protein (APP). The collaborative study showed that this motif enables APP to regulate the maintenance of mChol level especially at synapse, the communication node of neuronal network. Point mutations within this motif led to abnormal surface localization of APP, significant reduction of mChol, profound synaptic dysfunction, and progressive neuronal loss. Moreover, direct deprivation of mChol on top of those point mutations induced AD-like neuropathology. Based on findings, research staff postulate that the homeostasis of neuronal mChol is critical for neuronal function, and that its unbalance contributes to AD.

As the blood--brain barrier greatly restricts the access of pharmacological and genetical interventions, staff will use cultured rodent neurons and human neurons derived from induced pluripotent stem cells to test therapeutic strategies that mitigate neuronal dysfunction and loss by balancing mChol. To enable high--throughput assay, staff will employ novel cell imaging tools developed by staff. Here, researchers propose three Specific Aims. First, staff will establish *in vitro* preparations for mouse and human neurons that are robust enough for pharmacological and genetic manipulations. Second, staff will develop new fluorescent reporters and imaging--based measurements for neuronal activity, synaptic cholesterol, and neurodegeneration. Third, staff will test pharmacological, genetic, and nanomaterial-based approaches to restore mChol and neuronal health. The outcome of this project will provide new direction to slow down or even reverse AD in aging brain.

18. **Grant #20A18:** Impact of Cerebrovascular Pathology on Alzheimer's Disease and Other Dementia

    Principal Investigator: Saeid Taheri, PhD

    Organization: University of South Florida

    Abstract of Proposed Research: For the aging patient, there is a broad range of factors that may contribute to age--related cognitive impairment (CI). Diversity in CI signs and symptoms is believed to stem not only from the patients' cognitive reserve but also from the type of associated pathologies, such as vascular pathologies. Recent observations reveal that various cerebrovascular pathologies are implicated in Alzheimer's disease (AD) and other types of dementia. In most cases, multiple pathologies coexist that vary across the human aging spectrum. The manifestation of these pathologies can be observed via various biomarkers, including behavioral and psychological symptoms, *in vivo* imaging biomarkers, cerebrospinal fluid (CSF), and positron emission tomography (PET) markers such as specific AD biomarkers. However,

these behavioral and psychological symptoms have different prevalence, onset and course that make them even more unreliable in diagnosis application. A clear example is the variation of AD-CSF biomarkers. Therefore, there is a need to employ a more resilient biomarker that corresponds to the disease progression. In this case, cerebrovascular biomarkers could be more resilient with time. Therefore, researchers hypothesize that longitudinal epidemiological studies of cerebrovascular pathologies are better markers in classifying CI patients for different therapeutic options. Staff plan to test this hypothesis within two following specific aims. Specific Aim #1. To recruit CI patients with a history of vascular injury visible on MRI, and to investigate the current state of disease with respect to cerebrovascular injury. Rationale: Cerebrovascular injury manifests as an ill-perfused brain. Hypoperfusion, impaired cerebrovascular reactivity, and impaired BBB integrity are frequently observed in patients with various types of cognitive impairment. These symptoms are observed along with increased amyloid--beta (Aβ) load and impaired neuronal functions. Although there has been no confirmation that coexistence of cerebrovascular injury and AD markers implies cause and effect relations, Aβ accumulation may be impacted by cerebrovascular injuries. Specific Aim #2. To elucidate that the onset and course of cerebrovascular diseases impact CI severity. Rationale: The underlying pathogeneses of cognitive impairment manifest with different behavioral and psychological symptoms. These behavioral and psychological symptoms evidence different prevalence onsets and courses. Therefore, noting the course, onset, and pattern of behavioral and psychopathological features of CI are critical to identifying proper clustering of each CI subtype. Moreover, the temporality of a new clinical cerebrovascular event with new CI is an important association that could be used to cluster patients with CI. For example, extensive biomarker investigation is not needed to diagnose a CI subtype when a new stroke is immediately followed by CI.

To achieve these specific aims, staff plan to recruit 40 PET--positive and 40 PET--negative CI patients and investigate the health of cerebro-vasculatures by using advanced MRI techniques and correlate with blood/CSF biomarkers of cerebrocardiovascular disease and inflammation. Potential recruits would be from Byrd institute and VA center.

Impact: Classifying AD and other dementia patients based on the underlying cerebrovascular pathology may help in more specific therapeutic options which may help slow down the progression of AD and other dementias.

19. **Grant #20A19:** Lifestyle Stressors of Hippocampus and AD Related Brain Regions: Potential for Intervention

    Principal Investigator: Noam Alperin, PhD

    Organization: University of Miami

    Abstract of Proposed Research: Progression to Alzheimer's disease (AD) and

dementia from cognitively intact status is associated with accelerated brain tissue loss primarily in the medial temporal lobe. These critical regions demonstrate higher vulnerability to external stressors than other brain regions. Research staff are a multidisciplinary team of investigators with a track record in novel cognitive assessment for early detection of AD, sleep assessment, and in neuroimaging. Researchers aim to further assess the role of poor sleep quality in the symptomatic phase of the disease. Staff also propose to test the potential impact of possible countermeasures. This proposal builds on important findings staff made during a previously FDOH-funded project where staff compared volumes of amnestic mild cognitive impairment (aMCI)susceptible brain regions in cognitively-intact, good- and poor-sleeper elderly subjects (Alperin et al 2019 SLEEP). Staff found that the volumes of the hippocampus, amygdala, and superior parietal regions were significantly smaller (about 7.5% on the left side and 5-7% on the right side) in the cognitively-intact poor sleepers. Considering the large differences in the volume of these regions, the similarity in percentage loss is quite intriguing. Staff further found that the differences in the volume of these regions between good and poor sleepers in the aMCI cohort were less prominent than in the cognitively-intact cohort. This implies that the most impact toward slowing down progression to dementia as it relates to brain tissue volume loss, is intervention in the early phase when subjects still remain cognitively intact.

The two main aims of the project are:

1) Assess the differences in the “rate” of volume loss in the cognitively associated brain regions between the poor and good sleeper using follow-up scans (already available through another study) taken approximately two years after the initial baseline scan. Researchers will compare the age effect with the sleep quality effect on these volumes. This data will be used for development of morphological references for identifying subjects at risk and for assessment of the impact of sleep intervention.
2) Assess the impact of two types of interventions to improve sleep quality. One is based on cognitive behavior therapy (CBT) and the other uses audio tones applied through a head--bend, which have shown improved sleep quality.

Cognitive behavior therapy:
CBT has been identified as the superior approach compared to standard pharmacotherapy in adults with sleep issues. The CBT therapy protocol consists of six sessions (an initial assessment and five treatment sessions). Treatment begins with a comprehensive sleep assessment, which is used to guide selection of CBT components. Treatment components include stimulus control, sleep restriction therapy, relaxation, and cognitive therapy. The final session focuses on maintenance of gains and relapse prevention. This investigation not only will assess the outcome in the cohort but will provide the greatly needed imaging evidence to support the validity of the intervention.

SmartSleep Deep Sleep Headband:
This device, which was recently selected by NASA to improve astronauts sleep quality, utilizes a head band to deliver a variety of auditory stimulation protocol. The advantage

of this device is its ease of use and accessibility. The study will determine if this device indeed affects sleep quality in cognitively-intact elderly subjects by lowering the rate of brain volume loss measured in aim 1.

20. **Grant #20A20:** Between Here and There: Addressing End-of-Life Disparities Among African Americans with Mild Cognitive Impairment and Dementia Through Community-Based Training in Advance Care Planning

    Principal Investigator: Maisha Robinson, MD, MS

    Organization: Mayo Clinic Florida

    Abstract of Proposed Research: Although advance care planning in the United States in general occurs less frequently than it should, there is a notable difference in the completion of advance directives based on race/ethnicity that raises a specific, further concern about end--of--life disparities. Advance care planning is significantly less common in African Americans than in Caucasians as measured by the completion of advance directives. Primarily qualitative research suggests that the barriers to advance care planning among African Americans include low knowledge about the value of end--of--life care planning, mistrust of the healthcare system, an emphasis on communal decision--making relative to individual autonomy, and religious beliefs.

    Advance care planning is the process of making decisions regarding an individual's preferences for care at the end-of-life in the event that the individual is unable to communicate their wishes. It involves engaging in discussions regarding goals and preferences for end--of--life care, identifying a health care surrogate and completing an advance directive in an effort to respect autonomy at the end of life. African Americans are more likely than Caucasians to choose life--sustaining treatment at the end of life even if the burden of treatment outweighs the potential, limited benefits. Yet, African Americans are less satisfied than Caucasians with their care at the end of life.

    In African Americans with dementia, similar end--of--life care patterns are present with a lower likelihood of completing an advance directive and a higher likelihood of choosing aggressive, nonbeneficial invasive medical management at the end of life as compared to Caucasians. Modeled after a successful community--partnered pilot project focused on advance care planning among elderly, church- going African Americans, staff aim to refine and deliver a culturally relevant community education program on advance care planning for African Americans with mild cognitive impairment or dementia and their caregivers in a predominantly underserved African American community (New Town) in Jacksonville. Prior work by the principal investigator demonstrated that educational sessions delivered by trusted facilitators can improve knowledge about advance care planning, increase confidence to engage in discussions about end--of--life preferences, and increase readiness to complete advance directives. Moreover, the recent partnership with an African American neighborhood to implement a "dementia friendly community" model of dementia education and skill-training has shown that the

information presented within this model is retained better over longer periods of time than information presented through traditional outreach. Staff therefore plan to leverage this community--focused project to also develop and implement a “train--the--trainer” model to facilitate sustainability of the advance care planning program in the New Town community.

This intervention has the potential to improve end--of--life care planning and to reduce health care disparities at the end of life in a cohort of people who have a progressive condition with expected further cognitive decline, who will lose decision--making capacity, and who are disproportionately underutilizing advance directive documents.

21. **Grant #20A21:** Utility of Blood Biomarkers for Amyloid, Tau and Neurodegeneration to Assist in the Diagnosis of Alzheimer’s Disease and Other Dementias – Relationship to Cognition, Brain Atrophy and Amyloid Load

    Principal Investigator: Ranjan Duara, MD

    Organization: Mount Sinai Medical Center

    Abstract of Proposed Research: The pathology of Alzheimer's disease includes the formation of β-amyloid plaques (with deposition of two major forms of amyloid beta protein, i.e., aß 42 and 40) and the presence of neurofibrillary tangles (containing hyper-phosphorylated tau protein) in neurons. Synaptic loss and neuronal death, with the release of various proteins, especially Neurofilament Light (Nf-L), a biomarker of neurodegeneration, is a consequence of amyloid deposition and neurofibrillary tangle formation. Although aß 42 and 40 and Nf-L, assessed in the cerebrospinal fluid (CSF), have been in clinical use for many years and show excellent diagnostic accuracy. However, obtaining CSF samples by spinal taps poses an impediment to routine clinical use and to longitudinal studies. Recent development of ultrasensitive techniques for measuring aß42, 40, tau, and Nf-L in the blood have enabled use of the biomarkers for determining rate of disease progression in the general population. The ratio of Aβ42/40 in blood corresponds strongly to CSF measures of this ratio, with 89% accuracy for distinguishing amyloid positive from negative people. In preliminary studies among 350 participants from the 1 Florida ADRC, plasma Nf-L concentrations were found to be 2-3 times greater in those carrying a diagnosis of AD, Frontotemporal Dementia, and Vascular Dementia than in those with non-neurological diagnoses. Further, Nf-L concentration correlated strongly to the thickness of the entorhinal cortex and to Hopkins Verbal Learning Test scores.

    In this study, research staff will collect blood samples from 150 cognitively normal and MCI participants in the 1 Florida ADRC who come for annual follow--up evaluations and will obtain measures of aß42, aß4240, tau, and Nf-L (using Quanterix, Simoa HD-1 Analyzer). The objectives of this proposal are: (1) To assess the relationship of the concentration of a blood biomarker, using the assessing baseline measures of amyloid abeta 40 and 42, tau protein and neurodegeneration [by assessing Neurofilament Light

(Nf-L)] to the cognitive diagnosis (Normal, MCI, or mild dementia) and etiological diagnosis of MCI or dementia; (2) To evaluate the relationship of these biomarker concentrations to baseline measures of cognition (memory and non-memory), functional ability [using the CDR sum of boxes scale (CDRsb)], regional cortical thickness, and brain volumes and amyloid load measured on PET scans, accounting for age, sex, and ethnicity (Hispanic versus non--Hispanic); (3) To determine the predictive relationship of these biomarker concentrations to the rate of change in composite measures of memory and non-memory function, CDRsb scores and change in NPI scores; and (4) To assess the relationship of these biomarker concentrations to change in composite measures of memory and non-memory cognitive function and CDRsb over the previous 2-3 years, and then over the subsequent 2 years, accounting for age, sex, and Alzheimer versus non-Alzheimer diagnosis at baseline.

The long term goals of this study are to apply for federal funding to obtain longer term assessments with repeated blood biomarker and cognitive assessments to determine the utility of longitudinal biomarker assessments in tracking disease progression and potentially to response to treatment.

22. **Grant #20A22:** Racial and Ethnic Differences in Gene Expression Data

Principal Investigator: Rickey Carter, PhD

Organization: Mayo Clinic Jacksonville

Abstract of Proposed Research: Statewide collaborations have yielded the Florida Autopsied Multi--Ethnic (FLAME) cohort of patients. This cohort is comprised of autopsied brain tissue from a diverse population of Florida patients with neuropathologically--diagnosed Alzheimer's disease at time of death. This cohort contains Hispanic/Latino (n = 67), black/African American (n = 19), and white/European American (n = 1539) patient tissue. While the relative percentage of minorities is small, in absolute numbers, this cohort represents one of the world's largest minority Alzheimer's disease resources available for research. Early research on this cohort has found important differences in the characteristics of Alzheimer's disease between men and women and between age groups. Importantly, the pathology research on the cohort to date has expertly classified the disease burden and concluded there is widespread variability in the disease states in this cohort. Such differences may provide critical information into understanding what has been described as the "Hispanic Paradox" – the unexpected finding that, despite differences in socioeconomic status or vascular risk profiles and an increased risk for Alzheimer's disease, Hispanic Americans are found to live longer with the disease than European Americans. Uncovering mechanisms for this difference in prolonged survival is a significant research endeavor.

The objectives of this study are to: 1) obtain gene expression data on minority groups, and 2) assess differences in gene expression in Alzheimer's disease across ethno-racial groups. To achieve these objectives, first, we will conduct a robust examination of RNA

obtained from the donated brain samples using state--of--the--art equipment and best laboratory practices to determine how brain function is altered in the presence of Alzheimer's disease. This approach, which is known as "gene expression analysis", results in an abundance of data that requires highly skilled researchers to analyze and interpret the findings. Artificial intelligence approaches are now enabling these same scientists to gain new insights into complex disease processes such as Alzheimer's disease through more sophisticated analysis approaches. Our second aim for this research is to apply a previously--developed artificial intelligence model, which has been used on white/European American patient tissue, to our Hispanic/Latino and black/African American cohorts to directly test if the patterns of gene expression differ by ethno-racial groups. A final aim of this study will be to examine more general characteristics of Alzheimer's disease (e.g., how severe are the brain "tangles") by race and determine if any of these differences are explained through the gene expression data.

This research represents an important opportunity to study, in a detailed manner, how Alzheimer's disease varies by race and ethnicity. The results of our research will be broadly shared with hopes of stimulating new discoveries and potentially providing a means to have more refined diagnoses in patients with early signs of Alzheimer's disease.

Appendix B
FISCAL YEAR 2019-2020 ACTIVE GRANTS

(Funding Year 2018-2019)

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditures	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-On Funding
9AZ01	Florida Atlantic University	Rosselli, Monica	$ 235,018.60	$29,377.00	$205,641.60	05/05/2019	03/31/2021	No	No	No
9AZ02	Florida Atlantic University	Van Praag, Henriette	$ 237,500.00	36,458.00	213,542.00	03/01/2019	02/28/2023	No	No	No
9AZ03	Florida Atlantic University	Tappen, Ruth	$ 237,500.00	$20,833.00	$229,167.00	03/26/2019	04/30/2021	No	No	No
9AZ04	Florida Atlantic University	Galvin, James	$ 94,709.30	Relinquished						
9AZ05	Florida Atlantic University	Ghoraani, Behnaz	$ 95,000.00	$15,784.00	$78,925.30	03/01/2019	02/28/2021	No	No	No
9AZ06	Florida Atlantic University	Wei, Jianning	$ 94,998.82	$27,708.00	$67,292.00	03/01/2019	02/28/2021	No	No	Yes
9AZ07	Florida International University	Burke, Shanna	$ 237,500.00	$27,706.00	$67,292.82	02/20/2019	02/28/2021	No	No	No
9AZ08	Mayo Clinic Jacksonville	Ebbert, Mark	$ 237,500.00	$46,179.00	$191,321.00	02/07/2019	02/28/2022	No	Yes	No
9AZ09	Mayo Clinic Jacksonville	Li, Yonghe	$ 95,000.00	$26,518.60	$68,481.40	02/21/2019	02/28/2021	No	No	No

9AZ10	Mayo Clinic Jacksonville	Springer, Wolfdieter	$ 87,181.82	$50,855.00	$36,326.82	02/28/2019	02/29/2020	No	No	No
Grant #	**Organization**	**Principal Investigator**	**Award Amount**	**Life to Date Expenditures**	**Unspent Funds**	**Executed Date**	**End Date**	**Patents**	**Publications**	**Follow-On Funding**
9AZ11	Mount Sinai Medical Center	Greig-Custo, Maria	$ 237,500.00	$69,272.00	$168,228.00	04/01/2019	03/31/2021	No	No	No
9AZ12	University of Central Florida	Wharton, Tracy	$ 94,998.91	$27,706.00	$67,292.91	02/14/2019	02/28/2021	No	No	No
9AZ13	University of Florida	Garvan, Cynthia	$ 95,000.00	$15,840.00	$79,160.00	02/25/2019	02/28/2021	No	No	No
9AZ14	University of Florida	Maraganore, Demetrius	$ 237,500.00	$39,600.00	$197,900.00	02/26/2019	02/28/2021	No	No	No
9AZ15	University of Florida	Smith, Glenn	$ 237,500.00	61,353.40	176,146.60	05/07/2019	02/28/2021	No	No	No
9AZ16	University of Florida	Mitchell, Gordon	$ 237,497.89	$35,622.00	$197,917.89	03/28/2019	02/28/2021	No	No	No
9AZ17	University of Florida	Giasson, Benoit	$ 237,500.00	$39,500.00	$198,000.00	03/12/2019	02/28/2021	No	Yes	No
9AZ18	University of Florida	Weisbrod, Neal	$ 87,181.82	$16,355.50	$70,846.32	03/08/2019	02/28/2021	No	No	Yes
9AZ19	University of Florida	Price, Catherine	$ 237,080.10	$29,635.00	$207.445.10	05/06/2019	03/31/2021	No	No	Yes
9AZ20	University of Miami	Govind, Varan	$ 87,181.82	$58,120.00	$29,061.82	02/25/2019	02/29/2020	No	No	No

9AZ21	University of Miami	Curiel, Rosie	$ 84,301.00	$49,175.00	$35,126.00	02/20/2019	02/29/2020	No	No	No
9AZ22	University of Miami	Harvey, Philip	$ 87,830.00	$51,233.00	$36,597.00	02/18/2019	02/29/2020	No	No	No
Grant #	**Organization**	**Principal Investigator**	**Award Amount**	**Life to Date Expenditures**	**Unspent Funds**	**Executed Date**	**End Date**	**Patents**	**Publications**	**Follow-On Funding**
9AZ23	University of Miami	Brown, Scott	$ 95,000.00	$27,708.00	$67,292.00	02/21/2019	02/28/2021	No	No	No
9AZ24	University of Miami	Loewenstein, David	$ 237,171.38	$69,174.00	$167,997.38	02/25/2019	02/28/2021	No	No	No
9AZ25	University of Miami	Rundek, Tatjama	$ 237,500.00	$69,269.00	$168,231.00	02/21/2019	02/28/2021	No	No	No
9AZ26	University of South Florida	Dobbs, Debra	$ 237,496.20	$41,666.00	$208,300.00	04/04/2019	02/28/2021	No	No	No
9AZ27	University of South Florida	Conner, Kyaien	$ 95,000.00	$15,833.00	$79,167.00	04/04/2019	02/28/2021	No	No	No
9AZ28	University of South Florida	Meng, Hongdao	$ 94,860.35	$15,810.00	$79,050.35	04/04/2019	02/28/2021	No	No	No
9AZ29	University of South Florida	Webster, Jack	$ 95,000.00	$15,833.00	$79,167.00	04/17/2019	02/28/2021	No	No	No
9AZ30	University of South Florida	Bennett, Crystal	$ 94,991.99	$15,831.00	$79,160.99	05/10/2019	02/28/2021	No	No	No
9AZ31	University of South Florida	Gamsby, Joshua	$237,500.00	$26,389.00	$211,111.00	04/17/2019	03/31/2022	No	Yes	No

ACTIVE RESEARCH GRANTS FISCAL YEAR 2019-2020
(Funding Year 2018-2019)

1. **Grant #9AZ01:** Neuroimaging and Sensitive Novel Cognitive Measures in Detection of Early Alzheimer's Disease in Bilingual and Monolingual Hispanic Americans

   Principal Investigator: Monica Rosselli, PhD

   Organization: Florida Atlantic University

   Progress Report: This study has two main aims. Aim 1: To examine the extent to which bilingualism in amnestic Mild Cognitive Impairment (aMCI) is associated with: a) fewer deficits in the failure to recover from Proactive Semantic Interference (frPSI) on the LASSI-L (a cognitive stress test) and in other memory and executive function tests compared to monolingual Spanish speakers, and b) structural integrity of brain volumetric biomarkers in Alzheimer's Disease-prone regions in a large cohort of Hispanics with aMCI. Aim 2: To be the first study to determine the influence of bilingualism on the integrity of white matter tracts in aMCI, comparing Spanish/English bilinguals with monolingual Spanish speakers. This study will examine bilingualism, a cognitive stress test, and unique brain biomarkers in grey matter brain regions and white matter tracts in a sufficiently large sample to identify the individual contribution of variables such as education and acculturation. The most significant accomplishments of this project so far have been the recruitment of a significant number of Hispanic participants who are either Spanish monolingual or Spanish/English bilinguals and who meet Magnetic Resonance Imaging (MRI) inclusion criteria. This newly recruited sample will add to an existing sample of Hispanics at the Alzheimer's Disease Research Center with existing evaluations. A neuropsychological battery and an MRI scan will be done with all participants. Neuropsychological and neuroimaging data will be analyzed at FAU and UF, respectively. Results from these data will test the above hypotheses once research staff recruit a larger sample.

   Follow On Funding: None at the time of reporting.

   Collaboration: None at the time of reporting.

   Journals: None at the time of reporting.

   Patents: None at the time of reporting.

2. **Grant #9AZ02:** The Role of Exercise-Induced Systemic Factors in Alzheimer's Disease.

   Principal Investigator: Henriette van Praag, PhD

   Organization: Florida Atlantic University

   Progress Report: Research staff have conducted initial experiments. Testing of wildtype male C57Bl/6 mice in the touchscreen system has been completed and

analysis of the behavioral data of this experiment is in progress. In addition, blood samples and brain tissue were collected from the mice. Quantification of the number of newly born hippocampal neurons is in progress. Furthermore, researchers have set up the mouse running wheel system with new wireless components, computer, and software in the vivarium and have validated this system with cohorts of mice housed with running wheels. Research staff have also completed pilot studies of the fine morphology of adult-born hippocampal neurons utilizing retroviral vectors expressing green fluorescent protein (GFP) and have performed stereotaxic surgeries to inject wildtype mice to evaluate labeling efficiency and reporter expression. Morphological characteristics of the newly generated neurons have been assessed utilizing confocal microscopy and Neurolucida360, and staff are analyzing the data. Staff are currently breeding the mouse model of Alzheimer's Disease (AD), the APPswe/PS1D9 transgenic mice, for behavioral and histological experiments. Research staff are in the process of producing and testing vectors expressing Cathepsin B for in vivo experiments in the AD mouse model.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

3. **Grant #9AZ03:** Fit2Drive: Development and Testing of a Driver Risk Predictor for Individuals with AD

   Principal Investigator: Ruth M. Tappen, EdD, RN, FAAN

   Organization: Florida Atlantic University

   Progress Report: This report addresses the work performed in the second three months of this funded study. Having completed staff recruitment, Institutional Review Board (IRB) approval, and development of the database on Research Electronic Data Capture (REDCap), the primary task in this second quarter was to ramp up recruiting efforts, set appointments, and begin consenting and testing participants. This began in early July and has continued since then. Altogether, 17 participants have completed testing (an additional one failed to meet inclusion criteria and was not tested), six are scheduled for early November, and another 15 have agreed to participate and are being scheduled now. There is an additional group of eight from Belle Glade interested in participating, and transportation is being arranged to the Memory and Wellness Center in Boca Raton for the participants. In addition, a faculty member who recently received an National Institute of Health K award has connected the Project Coordinator with a network of places of worship from which staff can recruit participants. Staff also have been recruiting potential participants from the quarterly American Association of Retired

Persons (AARP) driving safety classes and from area senior centers. Response has been positive from both the organizations approached and the seniors contacted.

Some scheduling problems were encountered early in the quarter, finding that offices equipped with hardwire (Internet connectors preferable for REDCap data entry) were not available during participants' preferred appointment times. A meeting with the study Principal Investigator (PI), the Center Director, and Project Coordinator to discuss this impediment led to assignment of office space dedicated to this study. This has allowed the Coordinator the flexibility needed to facilitate setting appointments. Participants preferred making appointments weeks in advance; this has been accommodated in the appointment scheduling.

Minor delays occurred due to the important Jewish holidays that fell in this quarter and the threat of hurricane Dorian. It also has taken considerable time to connect with community groups and organizations and build momentum in recruitment and enrollment activities. It is important to maintain the momentum achieved and continue outreach to additional organizations and community groups. The next target will be the many retirement communities in this catchment area and a major recruitment effort at the Memory and Wellness Center's Walk in the Mall event in two weeks.

At present, the on-road driving examiner from the Safety Council is quite busy. The Center Director has been directed to hire an additional examiner (in process). Dr. Rosselli is also training two additional psychometricians to increase availability over the entire week.

So far, the study has been well received by both organizations contacted and the individuals who are participating. The recruitment is steadily increasing. Preliminary data is expected for next quarter.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

4. **Grant #9AZ05:** Technology-based Systems to Measure Dual-task (motor-cognitive) Performance as a Biomarker for Early Detection of Alzheimer's Disease

   Principal Investigator: Behnaz Ghoraani, PhD

   Organization: Florida Atlantic University

   Progress Report: There were 39 patients identified with mild cognitive impairment (MCI) and 41 with Alzheimer's disease (AD) who had all the attributes of the dual-task assessments performed during their functional tests. Machine learning approaches were used based on support vector machine and decision tree algorithms to make a

prediction for the MCI and AD patients using data from the dual-task assessment performed on the computerized walkway. The algorithms achieve approximately 67% accuracy at this point. The next contribution will be improving the accuracy using variations of these machine learning methods. Next, staff will identify the important predictive attributes as the walking biomarkers and use those to develop methods that provide the same biomarkers using the data from sensor data instead of the computerized walkway. In addition, research staff developed the system to collect the subjects' movement data from sensors as the subject performs the dual-task assessments. Researchers collected data from six subjects in this quarter. Staff will continue data collection in the next few months.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

5. **Grant #9AZ06:** Effect of Neuronal Activity on Synaptopathy in Alzheimer's Disease using a Novel Multi-electrode Microfluidic Platform

   Principal Investigator: Jianning Wei, PhD

   Organization: Florida Atlantic University

   Progress Report: This project was awarded in March, 2019. The updated general audience abstract is described below:

   Synaptic dysfunctions are considered among the earliest pathogenic events that are correlated with learning and memory losses in Alzheimer's disease (AD). Identifying these pathological synaptic changes (synaptopathy) is crucial for revealing early interventions. Most efforts are directed to investigate how beta-amyloid and hyperphosphorylated tau affect synaptic functions. However, the effect of neuronal activity in AD synaptopathy remains largely unexplored. This is partially due to the lack of an appropriate platform to biochemically and spatiotemporally study synaptic changes at high resolutions under neuronal stimulations. Conventional neuronal culture approaches have limitations in selectively studying nerve terminals without affecting cell bodies. Since AD is a multifactorial disease, this aspect of study is crucial to understand how neuronal activity, the convergent target for genetic and environmental interactions, modifies the progression of AD. Researchers have developed a novel in vitro microfluidic platform to study synaptic functions. Microfluidic chambers can provide unique insight into the axonal compartments independent of the soma and enable researchers to study the spatial role of beta-amyloid and tau. The built-in microelectrodes in these chambers allows staff to investigate AD-related synaptic dysfunctions coupled with programmable neuronal stimulations.

In this pilot grant, research staff propose to use this novel platform to study how different patterns of neuronal activity (physiological vs. repeated stimulation) contribute to AD synaptopathy. While synaptopathy can be studied from different perspectives, the focus will be on axonal protein trafficking and turnover, which are spatiotemporally regulated by neuronal activity and remain largely unknown in AD pathology. Research staff hypothesize that protein trafficking/turnover in response to repeated synaptic activity are impaired in AD, which further contribute to local protein aggregates formation, leading to synaptic dysfunctions and losses. To achieve this, staff will seed primary hippocampal neurons prepared from postnatal day 1 (P1) 3xTg-AD transgenic AD and wildtype pups in the microfluidic chambers. 3xTg-AD transgenic mice are useful to study plague and tangle pathology associated with synaptic dysfunctions. Specifically, researchers propose to investigate activity-related synaptic protein trafficking (Aim 1) and synaptic protein turnover (Aim 2) in AD primary neurons. A combination of molecular, genetic, and biochemical approaches, and live cell imaging will be used to analyze synaptic protein trafficking/distribution in different compartments of neurons (Aim 1) and monitor protein/organelle turnover at synaptic terminals (Aim 2) under programmable neuronal stimulations. Researchers have successfully culture primary hippocampal neurons in the microfluidic chambers and showed that different electrical stimulation patterns affect beta-amyloid production. This will be further investigated in the next phase of studies.

Successful completion of these studies will provide new insights into activity-related synaptic changes in AD neurons. The acquisition of such knowledge is critical to understand early molecular pathology of AD in the context of neuronal activity, which is complex and of high interest for AD research. In summary, the proposed study and data obtained so far should evaluate the promise of modulating neuronal activity as an early novel therapeutic strategy in AD treatments.

Follow On Funding: National Institute of Health $403,909 (pending).

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

6. **Grant #9AZ07:** Shared Neuroanatomical Models of Psychiatric Conditions and Alzheimer's Disease Spectrum Disorders: The Effects of Depression, Anxiety, and Sleep Disturbance and Associated Changes in Brain Morphology Leading to Alzheimer's Disease.

   Principal Investigator: Shanna L. Burke, MSW, MPH, PhD

   Organization: Florida International University
   Progress Report: This project is in the beginning stages. During this grant period, research staff conducted the following activities:

   Initial IRB approval gained from the Institutional Review Boards at UF and FIU.

UF conducted literature reviews on machine learning methods in relation to neurological applications of specific machine learning methods. Specifically, the team looked at how and why other research studies had used support vector machines, support vector regression, and random forest classifiers in their work and how they dealt with the issues of feature selection, cross-validation, feature scaling, missing data, and model training data set split percentages.

June 2019 dataset obtained from the National Alzheimer's Coordinating Center, with expanded structural MRI variables.

UF has begun to construct the machine learning models in Python for support vector machine and support vector regression using test data. This work includes creating internal source code repositories and the beginning documentation for the models in preparation for receiving the results of the aim 1 analyses and June 2019 data sets.

UF has begun to work on the aim 2 confusion matrix analysis Python code that will be needed to evaluate model accuracy.

IRB renewal approved for FIU on 9/17/2019 (IRB-18-0357-CR01). This approval expires 10/09/2022.

The analysis for aim 1 has been completed and the results are currently being processed.

Follow On Funding: None at the time of reporting.

Collaboration: Florida International University is the lead University, in collaboration with University of Florida. One doctoral graduate assistant at FIU worked on the grant proposal. One Master's level graduate assistant in biostatistics is working on processing the output from Aim 1 and organizing the tables, methods, and results.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

7. **Grant #9AZ08:** Identifying Functional Mutations in Top Alzheimer's Disease GWAS Genes using Long-read Sequencing in Brain Tissue

   Principal Investigator: Mark T W Ebbert, PhD

   Organization: Mayo Clinic Florida

   Progress Report: During the first quarter of this grant, researchers submitted a new manuscript for publication to a top journal, *Genome Biology* (impact factor = 13.214), and it was accepted and published online in late May (http://bit.ly/camouflaged_genes). As researchers had hoped, the article has generated significant attention in the academic community, with >6500 article access and four citations in only five months. It has also received significant attention on Twitter, where Altmetric (score = 134)

estimates this paper is in the 99th percentile for articles of the same age (https://www.altmetric.com/details/60690541). The paper demonstrates how long-read sequencing technologies address what are commonly referred to as 'dark' genomic regions. These 'dark' regions are stretches of DNA that standard short-read sequencing approaches cannot accurately assess. This work demonstrates the magnitude of the problem, identifying nearly 6000 genes that are at least partially 'dark' using short-read sequencing technologies. Long-read sequencing from PacBio (commercial product by Pacific BioSciences) closes more than 50% of these regions. While long-read sequencing technologies are a great solution moving forward, there are already many large sequencing studies, including Alzheimer's disease, that have sequenced thousands of individuals using short-read technologies. Research staff developed a computational method that can resolve many of these 'dark' regions in short-read data. Staff were able to rescue >4200 mutations that have been overlooked in the largest Alzheimer's disease sequencing dataset that exists (~13000 individuals). Rescued mutations included a rare ten-nucleotide frameshift deletion in CR1, a top-10 Alzheimer's disease gene. This mutation causes a loss of function of the CR1 protein, and currently segregates perfectly with disease, found in only five cases and zero controls. While staff cannot formally assess the mutation's involvement in disease from this dataset alone (insufficient sample size), researchers have obtained data from other large consortia and are currently screening those datasets. The work is going very well.

Dr. Ebbert has given six invited talks on this work, including one last month at the American Society of Human Genetics (ASHG) conference. ASHG is the largest human genetics conference in the world, and there were >300 attendees to the presentation. Dr. Ebbert was also invited to give another talk at the University of Florida in November, and gave an invited world-wide webinar in early October (http://bit.ly/camo_webinar).

Research staff are also starting to sequence individuals. Staff have sequenced 34 individuals and have demonstrated that the team can resolve some of the largest, most complex structural DNA mutations known. These regions are known to be involved in Alzheimer's disease. Staff also discovered new structural mutations in these regions and are ramping up efforts to determine whether these are associated with disease. Dr. Ebbert was invited to give two talks on this at ASHG during this reporting period. Because of the funds from this grant, researchers are beginning to sequence a larger cohort.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: M. T. W Ebbert, T. D Jensen, K Jansen-West, J. P Sens, J. S Reddy, P. G Ridge, J. S. K Kauwe, V Belzil, L Pregent, M. M Carrasquillo, D Keene, E Larson, P Crane, Y Asmann, N Ertekin-Taner, S. G Younkin, O. A Ross, R Rademakers, L Petrucelli, and J. D Fryer. Systematic analysis of dark and camouflaged genes reveals disease-relevant genes hiding in plain sight. *Genome Biology*, 20(1):97, 2019.

Patents: None at the time of reporting.

8. **Grant #9AZ09:** Therapeutic Roles of Surrogate Wnt Agonist in Alzheimer Disease

   Principal Investigator: Yonghe Li, PhD

   Organization: Mayo Clinic Florida

   Progress Report: Overproduction of the protein beta-amyloid is strongly associated with Alzheimer's disease (AD), but many drugs targeting beta-amyloid fail in development. Scientists are searching for novel therapeutic targets for AD prevention and treatment. Wnt/β-catenin signaling is an essential pathway that regulates numerous cellular processes, including neuronal survival. Critically, Wnt/β-catenin signaling is greatly suppressed in AD brain, and deregulated Wnt/β-catenin signaling plays an important role in the pathogenesis of AD. In this project, research staff will determine the therapeutic roles of a newly developed Wnt activator in AD. Successful completion of current study will allow staff to provide the rationale for activation of Wnt/β-catenin signaling as a new therapeutic strategy and identify the Wnt activator as a promising candidate to be developed for AD prevention and treatment.

   Follow On Funding: None at the time of reporting.

   Collaboration: None at the time of reporting.

   Journals: None at the time of reporting.

   Patents: None at the time of reporting.

9. **Grant #9AZ10:** Validation of Novel, Selective Autophagy Biomarkers in Alzheimer Disease

   Principal Investigator: Wolfdieter Springer, PhD

   Organization: Mayo Clinic Florida

   Progress Report: Alzheimer’s disease (AD) is the most common neurodegenerative disorder affecting more than 5 million individuals in the US with close to 500,000 in Florida alone. Clinically, AD is characterized by severe cognitive, behavioral, and motor impairments resulting from progressive synaptic dysfunctions and neuronal loss. Neuropathologically, AD is defined by the formation of insoluble protein aggregates including extracellular amyloid-β (Aβ) plaques and intracellular tau tangles. In addition to these late-stage hallmarks, mitochondrial dysfunctions and impairments of the autophagy-lysosome system are well-documented early signs of AD. Mitochondria (the cellular power houses), autophagy (the cellular garbage collection), and lysosomes (the cellular waste disposal and recycling system) are dynamic and vital organelles that are particularly important in neurons for their development, function, and survival. Emerging findings suggest an intimate interrelationship between them and it is now evident that

dysfunctions in either organelle results in impairment of the others. Those dysfunctions not only prominently occur in the prodromal phase of AD, but also further promote the accumulation of Aβ and tau through increase in oxidative damage, cellular energy deficits, and progressive failure of cellular degradation. To prevent accumulation of damaged organelles and proteins, cells employ several pathways of general and selective autophagy such as mitophagy (clearance of failing or worn out mitochondria), aggrephagy (clearance of protein aggregates), and secretory autophagy (release of intracellular material to the outside). Individual forms of selective autophagy are regulated through combinations of post-translational modifications that are typically transient unless flux through the clearance arms is impaired. These specialized modifications not only label the respective cargo (such as the 'mitophagy tag' phosphorylated ubiquitin), but also modify the autophagy receptors (such as p62/SQSTM1) that decode the signals and facilitate routing of material within the autophagy-lysosome system. Researchers capitalized on these findings and quantified levels and distribution of those selective autophagy markers in larger cohorts of human post-mortem brain samples from AD patients. Compared to controls, staff found significant increases of distinct autophagy labels and receptors that correlated with the respective neuropathologies. In addition to immunohistochemical analyses staff developed highly sensitive ELISA-type assays on a Mesoscale discovery platform and confirmed the utility of these markers as quantitative measures of mitochondrial damage, protein aggregation, and/or impairments of selective forms of autophagy. Here, researchers have begun to validate their potential as a biomarker (panel) in blood plasma samples from age and sex-matched neurologically normal controls, individuals with mild cognitive impairments, and patients with AD. The goal is to leverage existing, larger collections of plasma and CSF samples to correlate selective autophagy with the progression of disease from mild cognitive impairment to dementia.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

10. **Grant #9AZ11:** Impact of the MindSight Training Program on Patients with MCI and Early Stage Dementia

    Principal Investigator: Maria T Greig-Custo, MD

    Organization: Mount Sinai Medical Center

    Progress Report: During the period from July 1, 2019 to September 30, 2019, this study has focused on recruitment of eligible participants and their caretakers to the study. Mount Sinai Medical Center is situated in the Miami Dade area where a large and elderly Hispanic population of South Florida lives, therefore to include underrepresented

minorities in Alzheimer's research the informed consent forms, the study classes, and the neuropsychological testing and interviews have also been translated into Spanish. After participants were randomized into two modalities of group intervention, the Mindsight Group and the Attention Control intervention, the English speaking groups have already started for Mindsight group and in January the Attention Control groups will start . Also in January staff will start the Spanish speaking groups. The personnel are organizing consecutive groups to be held from January onwards. This has directed most of the present effort to recruitment. As projected in the grant, saliva samples have been collected in all the participants following the established protocol and a first batch of samples will be sent in December for its analysis.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

11. **Grant #9AZ12:** The Florida REACH Translation Project: Translating an EBP for an Outpatient Clinical Setting to Reach Diverse Community Members

    Principal Investigator: Tracy Wharton, PhD, MSc, MEd, MSW, LCSW

    Organization: University of Central Florida

    Progress Report: Few tested dementia caregiving interventions have been translated to practice. Translational research bridges a gap from practices that have shown efficacy to implementation in practice settings where feasibility and effectiveness can be tested. This pilot study aims to test feasibility and effectiveness of a modified Resources for Enhancing Alzheimer's Caregiver Health (REACH) II intervention, implemented in an outpatient clinic providing specialty care to diverse families, connected to the AdventHealth Memory Disorder Clinic. This study adapts critical components of REACH II: safety, well-being, skills development for ADL/IADL/behavior response, and material on care coordination, grief, and advanced care planning. It addresses the behavioral and social needs of persons with AD and their caregivers. The intervention comprises six sessions over 6-8 weeks, and is being provided in the clinic setting, linking the psychosocial intervention and medical team, and providing a foundational training for the family. By partnering with a medical clinic that serves a diverse community, staff hope that knowledge from this study will help improve care management of persons with AD and lead to transformative advances in how care is delivered to patients and their caregivers.

    As of this report, the intervention has been successfully translated for an outpatient setting, all manuals and materials have been completed in both English and Spanish language, and a strong partnership between the university and hospital research teams

has been developed. Staff have successfully completed intakes of the first four participants and recruitment is underway for potential participants in the study. The research team will present about the translation and implementation experience at the national annual Gerontological Society of America conference in November 2019. While implementation of this type of intervention in this setting has a number of challenges, these appear to be surmountable and the intervention appears to be feasible. Feasibility is the first aim of the project and provides the potential for access to a previously underserved demographic.

Follow On Funding: None at the time of reporting.

Collaboration: University of Central Florida is the receiving institution for the grant. Three undergraduate students and one graduate student so far have been involved in preparing for implementation of this work. The students have completed human subjects training, education about dementia and Alzheimer's disease, and training in grant management and submission of IRB documents.

These students are as follows:
Felicia Bender, undergraduate social work (BSW) student; University of Central Florida School of Social Work, Orlando, FL.

Angie Moliere, Health Sciences undergraduate student; University of Central Florida College of Health Professions & Sciences, Orlando, FL.

Lorrianne Brooks, Health Sciences undergraduate student; University of Central Florida College of Health Professions & Sciences, Orlando, FL.

Nicholas James, Clinical Psychology doctoral student; University of Central Florida Department of Psychology, Orlando, FL.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

12. **Grant #9AZ13:** Is Cortisol Really a Factor in Cognitive Decline?

Principal Investigator: Cynthia Garvan, PhD

Organization: University of Florida

Progress Report: The study team and roles:

Carlos Quinonez, Jr. (Research Coordinator, Mt. Sinai Medical Center, Miami)

Carlos recruits participants, obtains informed consent, acquires hair samples, distributes gift cards, and mails samples to the University of Florida.

Sara Harris (Research Coordinator, University of Florida)

Sara is the Study Team liaison to the University of Florida IRB and is responsible for assembling kits for obtaining hair samples. Sara is also responsible for reimbursing participants with $25 gift cards. Carlos mails hair samples to Sara, who delivers them to the Golde Lab at the University of Florida for processing.

Warren (Billy) Barker (Research Coordinator, Mt. Sinai Medical Center, Miami)

Billy is the Study Team liaison to the Mt. Sinai Medical Center, Miami IRB. Billy and Sara work together to coordinate implementation of the study according to the Mt. Sinai Medical Center and University of Florida IRB protocols.

Hunter Futch (MD-PhD student, University of Florida)

Hunter will participate in manuscript writing.

Bruce Goldberger, PhD (Co-Investigator, University of Florida)

Dr. Goldberger is the scientist who is directing the lab analysis of the hair samples.

Maria Greig-Custo, MD (Co-Investigator, Mt. Sinai Medical Center, Miami)

Dr. Greig-Custo, clinician, oversees the study operations at Mt. Sinai Medical Center and will work to recruit study participants.

Cynthia Garvan, PhD (Principal Investigator, University of Florida)

As Principal Investigator, Dr. Garvan, oversees all study operations and is responsible for full implementation of the study and adherence to IRB protocols. Dr. Garvan serves as study biostatistician, will conduct the statistical analysis of study data, and will direct dissemination of study results in presentations and manuscript(s).

TBA

Due to medical rotations, Dr. Hunter Futch will not be able to process samples. Dr. Goldberger is identifying a technician for sample processing.

Progress:

a. Kits and gift cards are in Miami.
b. Carlos Quinonez has recruited 7 participants from the Aging and Disability Resource Center (ADRC) cohort since Oct 17, 2019, and obtained informed consent and hair samples from six participants. The current rate of participant recruitment puts researchers on track for timely study completion.
c. Dr. Goldberger has examined one of the samples to check for compliance with protocol. Dr. Goldberger found the sample to be expertly obtained with sufficient volume of hair for assay.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

13. **Grant #9AZ14:** Utilizing Data from the Electronic Medical Record to Predict Alzheimer's and Dementia Risk

    Principal Investigator: Demetrius M Maraganore, MD

    Organization: University of Florida

    Progress Report: This project aims to utilize data from the electronic medical record (EMR) to predict Alzheimer's and dementia risk. Predicting and preventing Alzheimer's disease (AD) is a major public health priority. One in five women and one in 10 men residing in Florida will develop AD in their lifetime. Presently there are 560,000 Floridians with AD, and that number will grow nearly 30% over the next five years (projected prevalence, 720,000 cases by 2025). AD destroys the lives of patients, burdens caregivers, and is financially devastating. This project, if successful, will build and implement an EMR-based data infrastructure to predict and prevent AD in Florida, engaging as many as 22 hospitals and health systems, 1,240 practices, 4,100 providers, and 15 million patients statewide (the OneFlorida Clinical Research Consortium): https://onefloridaconsortium.org/. Researchers are leveraging available information from the OneFlorida Data Trust to fulfill the following aims: 1) to build an AD prediction model at the University of Florida (UF); 2) to replicate the model across the remaining OneFlorida sites; 3) to build clinical decision support (CDS) tools into the UF EMR that alert primary care providers of the patients at greatest risk for AD; and 4) to share the AD prediction model and CDS tools with the remaining OneFlorida sites.

    With respect to the most significant scientific accomplishments to date, research staff have largely completed Aim 1 of the grant (Model Building): Use data captured by the UF EMR to develop an AD and related disorders prediction model. Research staff identified a historical cohort at UF from whom AD prediction model are built. Staff defined the dependent and independent variables needed for the model building and explored two distinct methodological approaches. The first approach is hypothesis driven: the model is constrained to independent variables that are discretely captured by the EMR and that have a priori evidence for an association with AD, and the analytic method is stepwise multiple regression model building. The second approach is hypotheses free: the model allows all independent variables that are discretely captured by the EMR to be included (regardless of prior associations with AD or not), and the analytic method is machine learning. Researchers are now preparing to enhance the first two models by exploring the added value of natural language processing (NLP) of free text: to capture additional instances of the dependent or independent variables (that may have escaped capture as discretely coded data), and to include the NLP-captured data into the prediction models. Once researchers have completed the NLP processing steps,

staff will identify the AD prediction model (hypothesis driven, hypothesis free, or either model enriched by NLP) that best fits the UF data, and move that model forward to Aim 2 . Staff expect to transition to Aim 2 by the end of 2019.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

14. **Grant #9AZ15:** Association of PET Amyloid Status with Cognitive and Functional Outcomes of Behavioral Interventions in Mild Cognitive Impairment

    Principal Investigator: Glenn Smith, PhD

    Organization: University of Florida

    Progress Report: This protocol involves the first-ever utilization of amyloid PET scanning for research at UF. During this reporting period research staff obtained IRB approval on 8/26/19 for this sensitive project which requires the handling of radiolabeled immunohistochemicals for PET scanning. Staff also obtained Office of Clinical Research review and mark up of the contract with the provider of the amyloid tracer Neuroceq®. Researchers sent a first round of 18 invitation letters to prospective participants and obtained initial positive responses from seven potential participants as well as four declinations, and are currently conducting follow-up calls for seven remaining initial prospective participants. Staff anticipate conducting the first series of scans in November 2019.

    Follow On Funding: None at the time of reporting.

    Collaboration: None at the time of reporting.

    Journals: None at the time of reporting.

    Patents: None at the time of reporting.

15. **Grant #9AZ16:** The Two Faces of Hypoxia in Alzheimer's Disease

    Principal Investigator: Gordon Mitchell, PhD

    Organization: University of Florida

    Progress Report: This new project is designed to test the idea that intermittent periods of low oxygen can either accelerate (high dose, chronic intermittent hypoxia simulating sleep apnea) or slow Alzheimer’s disease progression (low dose, therapeutic acute intermittent hypoxia) in a mouse model of Alzheimer’s disease.

Since the last progress report, postdoctoral associate Alex Marciante: 1) began collecting data on several cohorts of 2-month old mice exposed to two weeks of “high dose” intermittent hypoxia versus normal conditions; 2) will have approximately 100 samples ready for analysis by mid-November; and 3) has continued to genotype, breed, and age mice as appropriate for the current and future studies related to this project. These data will provide important information related to Aim 1 and aid in guiding next steps. Dr. Marciante has also: 4) continued to test the impact of different intermittent hypoxia protocols on native Tau phosphorylation state in the hippocampus and prefrontal cortex or normal rats; these tissues were already available and serve both to train Dr. Marciante in the relevant techniques, and advance understanding of intermittent hypoxia and Tau biology. Finally, Dr. Marciante 5) found that there is a region-specific & dose-dependent effect of intermittent hypoxia on Tau phosphorylation brain areas related to early-stage Alzheimer’s Disease, and is preparing an abstract based on these findings for presentation at the Experimental Biology Conference in April 2020. Results from these studies provide support for the central premise and help guide future experiments concerning “high dose” intermittent hypoxia effects on the development and progression of brain pathology related to Alzheimer’s Disease.

If the researcher’s ideas prove to be correct, this will be some of the first insights concerning two new therapeutic strategies to slow (avoid sleep apnea) or treat Alzheimer’s disease progression (therapeutic intermittent hypoxia). Given the large population of aging Floridians, Alzheimer’s disease is expected to reach epidemic proportions, with most individuals suffering from co-morbid sleep disordered breathing. This research addresses an important health concern for a highly prevalent disease that currently has no effective treatments to prevent or even slow the disease.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

16. **Grant #9AZ17:** Mechanisms of Abnormal Neuronal Tau Accumulation, Interactions with Amyloid-beta and Pathological Sequelae.

    Principal Investigator: Benoit Giasson, PhD

    Organization: University of Florida

    Progress Report: The presence of brain extracellular deposits of amyloid-beta peptides and the accumulation of neuronal aggregates comprised of the protein tau are defining hallmarks of Alzheimer’s disease (AD). The abundance and distribution of tau aggregates correlates with disease progression and clinical symptoms in AD. The direct

involvement of tau in disease has been unequivocally established by the discovery of tau mutations that results in progressive dementia. However, the mechanisms that lead to abnormal tau accumulation and whether pathogenic interaction between amyloid-beta peptides and tau lead to AD type neurodegeneration still remain unclear. Although tau is normally more abundant in the distal (axon) compartment of neurons, in AD it abnormally accumulates in the proximal compartments (cell body and dendrites). Numerous tau mutations that cause neurodegeneration affect tau's ability to interact with microtubules, a key component for its transport to neuronal distal component. Until recently, tau mutations were reported to only reduce interaction with microtubules, but new mutations have been identified that demonstrate the opposite property. In this project researchers propose to investigate the hypothesis that alterations in tau structure can lead to abnormal tau compartmentalization and that this process can be exacerbated by amyloid-beta deposition promoting neurodegeneration in rodent models of AD. Collectively, these studies will provide novel insights into the molecular and cellular mechanisms influencing the aberrant accumulation of AD protein deposits and the pathogenic consequences associated with tau aggregation with therapeutic implications.

Research staff have started to work on most of the studies described in the Specific Aims. Almost all of the DNA constructs for mammalian expression of tau proteins as well as DNA constructs for rAAV-mediated tau expression were generated. Staff also have initiated most of the studies using 293T cells proposed in Aims 1. Some of these data have been accepted for publication [Xia et al (2019) J. Biol. Chem. In press].

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: Xia, Y., Sorrentino, Z.A., Kim, J.D., Strang, K.H., Riffe, C.J., Giasson, B.I. (2019) Impaired tau-microtubules interactions are prevalent among pathogenic tau variants arising from missense mutations. J. Biol. Chem. In- press.

Patents: None at the time of reporting.

17. **Grant #9AZ18:** Responses to a Standardized Approach to Advance Care Planning in Cognitive Disorders Clinic

Principal Investigator: Neal J. Weisbrod, MD

Organization: University of Florida

Progress Report: This study seeks to pilot a standardized approach to advance care planning conversations in an outpatient neurological setting. Advance care planning (ACP) among patients with Alzheimer's disease and other forms of dementia poses unique challenges to clinicians. Clinicians often worry that having discussions about bad

outcomes or death will damage their relationship with patients and their families or cause undue anxiety or depression if not timed properly. This results in ACP being addressed after dementia has progressed and requires a health surrogate or proxy to make decisions. This study aims to address these barriers to ACP conversation by investigating the effect of a routine and standardized approach to discussing advance care planning in clinic. The Principal Investigator, a palliative-trained neurologist, will enlist patients diagnosed with cognitive impairment disorder or dementia who visit his clinic. The provider will guide advance care planning conversations with patients identified with mild or moderate dementia during their second office visit, approximately three months after the first visit. A control group of participants is recruited from another provider, who will not provide a structured ACP conversation. The study will compare the rate of advance directive (AD) upload between intervention and control group, in addition to studying patients' and caregivers' emotional attitudes using two assessments, Beck Hopelessness Scale (BHS) and Hospital Anxiety and Depression Scale (HADS), to quantify the effect of the intervention.

The study was IRB approved in June 2019 and underwent major revisions to maximize recruitment efficiency and eliminate deviations in the process of data collection between intervention and control provider. Recruitment effort is on going and refinement of the recruitment process will expand the reach to potential participants and speed up the recruitment process.

Follow On Funding: Florida Department of Health $87,181.82.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

18. **Grant #9AZ19:** Impact of Total Knee Replacement Surgery on Trajectory of Cognitive Decline in Individuals with Mild Cognitive Impairment (MCI)

    Principal Investigator: Catherine Price, PhD

    Organization: University of Florida

    Progress Report: In the recent NIH study following patients age 60 and older through knee replacement surgery with general anesthesia using the same surgeon and anesthesia protocol, researchers show that patients with mild cognitive impairment (MCI) have: 1) significant decreases in brain communication (functional network connectively) acutely after surgery (see Huang, et al, 2018); 2) less microstructural free water change acutely after surgery, suggesting preoperative brain integrity is an important contributor to a normal reactive inflammation response (Tanner, et al, 2019); and 3) greater intraoperative frontal EEG variability from time of tourniquet up to tourniquet down, suggesting desynchronization of neuronal networks with implications for cognitive insult (Hernaiz et al, 2019). These findings bring follow-up questions. *Why are patients with*

MCI's brains vulnerable to surgery and anesthesia? Should MCI patients have a different anesthetic approach? Are MCI patients who show acute brain changes after surgery more likely to have neuronal change even after one year? These are important questions that require investigation with longitudinal imaging and strategic focus on MCI recruitment. The current study involves prospective recruitment of individuals electing total knee replacement randomized to general anesthesia (n=14) versus regional anesthesia (n=14). Both groups acquire baseline MRI and acute 48-hour functional MRI using sophisticated structural, functional, and diffusion sequences. Each participant completes cognitive testing at a pre-surgery/baseline time point followed by repeat cognitive testing at 3-weeks, 3-months, and 1-year post-operative/post-baseline. To examine performance over time, a subset of participants (total n=14) will receive additional MRIs at 3-months and 1-year. Since study initiation, staff have completed research board approval, completed the MRI upgraded protocol, and initiated recruitment. The surgeon has referred 16 potential participants of which six participants are pending and one participant completed baseline and post-surgery MRI. Nine potential participants declined the study (reasons include dislike for the randomization approach, unable to handle the MRI, or difficulties with travel). The participant who enrolled in the investigation completed a pre- and post-operative MRI and was followed successfully through the surgical procedure without complications. Research staff are continuing to enroll participants as part of the planned investigation.

Follow On Funding: Philanthropic Donor - $25,000.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

19. **Grant #9AZ20:** Role of Gut Microbiota on the Brain Metabolism, Cognition, Immune Function and Inflammation in Alzheimer's Disease: Novel Biomarkers and Understanding Mechanisms

    Principal Investigator: Varan Govind, PhD

    Organization: University of Miami

    Progress Report: Despite identification of the hallmark pathological features of Alzheimer's disease (AD), which include extracellular amyloid beta plaques and intracellular hyperphosphorylated tau neurofibrillary tangles in the brain, the underlying causes and mechanisms contributing to these features remain largely unknown. Thus, the quest to identify the source of AD pathology continues to expand. Healthy gut microbiota or its metabolic products are known to be necessary for the maturation, activation, and optimal functioning of microglia, which are the resident macrophage cells that are responsible for scavenging plaques and infectious agents in the CNS. Most recently, dysbiosis of the gut microbiota (i.e., alterations in the microbiome diversity and

composition) has been shown to play a role in the pathogenesis of AD and other neurodegenerative diseases through the bi-directional communication pathway between the gut and brain (i.e., *the gut-brain axis*). Findings from several preliminary investigations indicated that the mechanisms contributing to the development of AD pathology may involve gut-microbiota-derived metabolites (e.g., GABA, a neurotransmitter; amyloid peptides and lipopolysaccharides); leaky guts; and inflammation. However, there is no published comprehensive data that examined associations between dysbiosis of the gut microbiome, concentration of brain GABA, markers of brain inflammation (myo-inositol - a marker of gliosis and free water fraction), concentration of peripheral amyloid peptides, markers of peripheral inflammation, and measures of neurocognitive function in patients with AD.

The goal of this study is, therefore, to test the associations between dysbiosis of the gut microbiome (i.e., diversity and composition) and intestinal permeability and microbial translocation markers with brain GABA, brain inflammation markers (myo-inositol and choline-containing compounds (Cho); free water content), peripheral amyloids and lipopolysaccharides, peripheral inflammation markers, and cognitive function in patients with cognitive impairment and in age-matched healthy controls. The specific aims include: 1) Compare the diversity and composition of gut microbial communities of stool samples from patients with cognitive impairment and healthy control using 16S rRNA-based metagenomics. *Hypothesis:* Te AD group will have a different microbiota diversity and composition than controls; and 2) Correlate gut microbial dysbiosis measures with severity of disease using blood-based peripheral measures (amyloid peptides, lipopolysaccharides, inflammation), MRI-technique based CNS measures (GABA, myo-inositol, Cho, free water content); and measures of neurocognition. *Hypothesis:* The gut microbial dysbiosis measures in AD group will have correlations with peripheral, CNS; and neurocognition measures and disease severity. The data obtained in this pilot study will enable researchers to identify novel biomarkers of AD and derive stable estimates of their variability across subjects for writing a standard grant application.

The Institutional Review Board (IRB) approval for this human subject study has been obtained. The research team has pre-screened patients seen at the Cognitive Disorder Clinics (University of Miami) within the last six months and short-listed 33 patients. Research staff will start enrolling 20 patients from this short-list who are willing to participate in this research study; the enrollment is scheduled to begin 10/14/2019. Staff hope to enroll the spouses or caregivers of these 20 patients (n=20) to serve as controls for this study.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

20. **Grant #9AZ21:** Postdoctoral Research Fellowship in Neuropsychology

Principal Investigator: Rosie E. Curiel, PsyD

Organization: University of Miami

Progress Report: Competent clinical assessment that is sensitive to detect preclinical AD remains a critical priority area in Alzheimer's disease research. Offering this training opportunity to a neuropsychologist is of particularly high impact, as this discipline plays a direct and critical role in Alzheimer's disease clinical research. The postdoctoral fellowship in Cognitive Neuroscience and Neuropsychology at the University of Miami Center for Cognitive Neuroscience and Aging (CNSA) is focused on providing specialty training in Alzheimer's disease and related dementias by: a) developing enhanced clinical evaluation and diagnostic skills, b) participating in ongoing clinical research projects that are studying promising new methodologies to improve the clinical assessment of diverse older adults at risk for the development of AD and related disorders, c) learning about cross-cultural neuropsychological assessment and the development of culturally fair diagnostic assessment instruments, which is of critical relevance in the State of Florida and d) receiving training in writing federally funded grant applications for preparations to become an independent investigator.

The regular academic cycle for postdoctoral fellows in neuropsychology begins in August-September of each year. In order to obtain the most competitive pool of candidates, the application period opened from the time of the notice of grant award in January, 2019 until March 31, 2019. This allows for a larger national pool of excellent qualified candidates who were seeking postdoctoral specialty training working with neurodegenerative diseases in a geriatric population. Research staff interviewed the top ten candidates based on history of academic achievement, focus of prior training, interests in developing clinical research skills as related to diseases of aging and cognition, endorsements from previous supervisors, and training goals that were in alignment with the mission and vision of early detection of AD+ADRD in a highly diverse population. Dr. Katherine Gorman, a Doctor in Clinical Psychology was selected to fill the training position. Dr. Gorman has submitted all institutional requirements and joined the University of Miami Miller School of Medicine on July 22, 2019.

The CNSA fellowship program was designed to train an exceptional candidate interested in postdoctoral specialty training in patient-centered AD clinical research with the unique opportunity to develop the skills to excel in the field of neuropsychology. Through the fellowship program, Dr. Gorman is receiving training in conducting evaluations, has been participating in the collection and analysis of AD related longitudinal data, and has been exposed to the administration and interpretation of state-of-the-art innovative assessments, and the data's correlation to AD biological biomarkers.

Dr. Gorman has successfully completed the training program's intensive orientation process that allowed familiarity with the multiple state and federally funded RO1 scientific protocols. This training included the clinical evaluation of older adults and the administration, scoring, and interpretation of a broad range of neuropsychological tests,

including traditional and novel assessments. In addition, Dr. Gorman is now CITI certified and has become a formal study team member on every IRB approved protocol. Dr. Gorman has been very enthusiastic, engaged, and integrated with the research team and has been participating on all required didactic trainings and weekly diagnostic consensus meetings. As such, research staff have received positive feedback from faculty members on Dr. Gorman's presentations and contributions. Dr. Gorman has been professional and seeks supervision as needed and has also shown above-average skills on the administration and interpretation of neuropsychological assessments, writing abilities, and diagnostic aptitudes. Dr. Gorman has also demonstrated leadership skills and has taken a role in mentoring pre-doctoral students and research associates. During the upcoming quarter, Dr. Gorman will be working on refining research ideas for the fellowship project and analyzing existing data for presentation at an upcoming scientific meeting.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

21. **Grant #9AZ22:** Postdoctoral Fellowship in Cognitive Neuroscience and Neuropsychology

    Principal Investigator: Philip Harvey, PhD

    Organization: University of Miami

    Progress Report: The postdoctoral fellowship in Cognitive Neuroscience and Neuropsychology at the University of Miami Center for Cognitive Neuroscience and Aging (CNSA) has been awarded to Dr. Christian Gonzalez, a bilingual and bicultural Doctor in Clinical Psychology. Dr. Gonzalez began the postdoctoral fellowship year at the University of Miami Miller School of Medicine on July 22, 2019.

    The regular academic cycle for postdoctoral fellows in neuropsychology begins in August-September of each year. In order to obtain the most competitive pool of candidates, the application period opened from the time of the notice of grant award in January 2019 until March 31, 2019. This provided a larger national pool of excellent, qualified candidates who were seeking postdoctoral specialty training working with neurodegenerative diseases in a geriatric population. Staff interviewed the top ten candidates based on history of academic achievement, focus of prior training, interests in developing clinical research skills as related to diseases of aging and cognition, endorsements from previous supervisors, and training goals that were in alignment with the mission and vision of early detection of AD+ADRD in a highly diverse population.

The fellowship program was designed to train an exceptional candidate interested in postdoctoral specialty training in patient-centered AD clinical research with the opportunity to develop the necessary skills to excel in the field of neuropsychology. Through the training program, Dr. Gonzalez is participating in the collection and analysis of longitudinal data and is receiving training in state-of-the-art innovative assessments, the latest biomarkers, and groundbreaking cognitive retraining interventions.

Dr. Gonzalez has successfully completed the training program's intensive orientation process that allowed familiarity with the multiple state and federally funded RO1 scientific protocols. Dr. Gonzalez is now CITI certified and is a formal study team member on every IRB approved protocol. In addition, Dr. Gonzalez has successfully cross-trained across protocols. This training includes the evaluation of older adults and the administration, scoring, and interpretation of a broad range of neuropsychological tests (traditional and novel) in both English and Spanish. Dr. Gonzalez has integrated well with the team and has participated in all required didactic trainings and diagnostic consensus meetings. Research staff have received feedback from other faculty that the presentations have been excellent and professional. Dr. Gonzalez has demonstrated above-average skill acquisition and competence. Dr. Gonzalez seeks supervision appropriately and is mentoring pre-doctoral students as well using a developmental model. During the upcoming quarter, Dr. Gonzalez will be working on conceptualizing the postdoctoral project and analyzing existing data for presentation at an upcoming scientific meeting.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

22. **Grant #9AZ23:** Impacts of Neighborhood Greenness & Greening Initiatives on Alzheimer's Disease in Medicare Beneficiaries

Principal Investigator: Scott C. Brown, PhD

Organization: University of Miami

Progress Report: This research project, newly awarded and executed in 2019, seeks to expand the knowledge base relative to Alzheimer's disease (AD) prevention through an investigation of the potential impact of the levels of greenness, such as tree canopy and general vegetative presence in neighborhoods. This study explores greenness as a new factor that could influence the impacts of AD. In prior 2010 analyses, the research team found that higher levels of greenness on neighborhood blocks were associated with lower rates of AD in Miami-Dade County Medicare beneficiaries. Subsequently, Miami-Dade County planted more than 200,000 trees in primarily low-income neighborhoods,

expanding countywide tree canopy from 14% of the county in 2010, to 20% of the county in 2016, creating the opportunity for this pilot project.

This project draws on this natural experiment of tree planting by the county to examine the relationship of greenness to the incidence of new cases of AD. The team is comparing AD diagnoses based on the International Classification of Diseases (ICD) codes used by the Centers for Medicare and Medicaid Services (CMS) in the records of 60,000, low-income Miami-Dade Medicare beneficiaries in 2010 and 2016, the pre- and post-tree planting period. The research team will assess all ~9,000 low-income Census blocks in Miami-Dade County to develop three categories. Category 1 identifies blocks that were low in greenness in 2010 and received no new tree plantings. Category 2 identifies blocks which already had a high level of greenness in 2010 and maintained that level in 2016. Category 3 identifies blocks that were low in greenness in 2010 and received tree-plantings to achieve a high level of greenness in 2016. Utilizing statistical matching techniques, the research team will randomly select 1,000 blocks in each of the three block categories and investigate the impact of greenness on incidence of AD. Based on the research team's 2010 study, the team hypothesizes that the original high greenness group might show consistent levels, the original low greenness group without the benefit of new plantings might show higher rates of AD, and the original low greenness group who received new planting might show fewer AD diagnoses than would have been projected without the plantings.

To date, the team has completed initial meetings with implementation partners at Miami-Dade Parks; acquired and processed the 2016 imagery to calculate greenness for all ~36,000 Census blocks in Miami-Dade County; and expanded the 2010 data set to include 2016 greenness levels and AD diagnoses. The team is now beginning to categorize the blocks and correlate with CMS data.

If initial results are promising, and after further validation in other Florida counties, this work could significantly contribute to the field of AD prevention/ treatment through multi-sectoral partnerships that increase access to greenness in neighborhoods. Given the benefits associated with greenness in relation to other disease burdens, as well as in the reduction of heat loads, energy usage, and other environmental benefits, greening represents a high return on a relatively modest investment.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

23. **Grant #9AZ24:** Middle-aged Offspring of Late Alzheimer's Probands: Novel Cognitive and Biomarker Assessment

    Principal Investigator: David A. Loewenstein, PhD

Organization: University of Miami

Progress Report: This study examines the underpinnings of the earliest manifestations of Alzheimer's Disease (AD) among a large group of well-defined middle-aged children who are offspring of one or more parents with late onset AD (O-LOAD) and will be compared to age and educationally equivalent controls without any family history of LOAD. This represent the first study conducted in the United States to evaluate a promising novel cognitive marker, the failure to recover from proactive semantic interference (frPSI) in at-risk O-LOAD. The study team will employ a combination of: a) a novel cognitive stress test, the Loewenstein Acevedo Scales for Semantic Interference and Learning (LASSI-L) uniquely sensitive to frPSI; b) fMRI measures of brain connectivity; c) structural MRI including diffusion tensor imaging (DTI); and d) genetic profile analyses. Sixty middle-age O-LOAD participants will be compared to age and educationally equivalent controls without any family history of LOAD.

During this reporting period, the group of investigators finalized the implementation of the scientific protocol and obtained IRB approval to start the study activities. The Principal Investigator (PI) hired a new Study Coordinator during the month of April 2019. The study coordinator completed all the required trainings during the month of May and started the outreach and recruitment activities in June. The first study participant was enrolled on July 03, 2019. During this reporting period, the study team enrolled 24 participants. Seventeen have completed Magnetic Resonance Imaging (MRI) scans. Research staff anticipate that 30 new participants will be enrolled in the upcoming reporting period (October-December 2019). The study team continues conducting outreach activities to recruit new study participants and staff are scheduled to advertise the study on our local public broadcast station. Six of the participants enrolled were deemed ineligible due to meeting exclusion criteria described in the protocol. While it is intended that every participant undergo a brain MRI, a few in this age group have been ineligible because of claustrophobia or implanted metal devices, thus staff plan to over-recruit to ensure study aims are met.

Continued investigation in this area will lead to critical scientific insights into the earliest pathogenesis of late onset Alzheimer's Disease (LOAD) and the obtained results should also have extremely important clinical and research implications for early cognitive screening, emerging treatment and prevention strategies. This project will also provide the necessary preliminary data to support a future longitudinal NIH funded study of middle-age O-LOAD participants.

Follow On Funding: None at the time of reporting.

Collaboration:

Postdoctoral Researchers: Three postdoctoral research fellows are currently collaborating in the research activities.

Nova Southeastern University, College of Psychology: Three doctoral students from the Clinical Psychology Program are assisting in the research activities as part of the practicum experience program.

Carlos Albizu University: One doctoral student from the Clinical Psychology Program is assisting in the research activities as part of the practicum experience program.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

24. **Grant #9AZ25:** Brain Vascular Imaging Phenotypes, Vascular Comorbidities and the Risk for Alzheimer Disease: The Florida VIP Study of AD Risk

    Principal Investigator: Tatjama Rundek, PhD

    Organization: University of Miami

    Progress Report: The overarching goal of this proposal is to determine the impact of novel brain vascular imaging phenotypes (VIPs) of small vessel disease and modifiable vascular comorbidities on cognitive and neurodegenerative profiles typical of Alzheimer disease (AD). Brain small vessel disease is the most prevalent cause of progressive cognitive impairment in the elderly. Brain imaging studies have shown the high prevalence of covert small-vessel disease in the elderly and population autopsy series have verified the high frequency of the coexistence of vascular pathology with AD pathology. The need for quantitative evaluations of the impact of brain vascular phenotypes on cognitive and neurodegenerative changes related to AD pathology is evident. To achieve project goals, staff will leverage in-depth brain magnetic resonance (MR) imaging, and clinical and neurocognitive data from the NIH-funded 1Florida Alzheimer Disease Research Center (1FL ADRC), which has enrolled a diverse population from South Florida with a large representation of Hispanics-Latinos. Available data in the 1FL ADRC include MRI and Amyloid PET scans together with demographics and clinical and neuropsychological data. The MRI data has been quantified for brain cortical thickness measures, which have already been used to identify the neurodegenerative changes (atrophy in selectively vulnerable brain regions) typical of AD. A major effort in this application will be to measure and quantify new phenotypes of brain vascular disease pathology from collected MRI scans, which has thus far been challenging, especially for small vessel disease, and has not reached a level of quantification similar to methods available for quantifying neurodegenerative changes. In the proposed study, researchers will utilize volumetric sequences from 300 MRI scans to create a VIP of small vessel disease, which will include silent brain infarcts, enlarged perivascular spaces, volumes of regional white matter hyperintensity volumes, and cerebral microbleeds. In addition, staff will develop a vascular comorbidity (VASCom) score utilizing data elements known to be associated with stroke and cardiovascular disease, from the extensive 1FL ADRC clinical database. Staff will then integrate the VIP of small vessel disease and VASCom score into a comprehensive model that will assess

their impact on cognitive performance that are sensitive to detect individuals at increased risk of AD, and that can assess the severity of neurodegeneration on MRI and the amyloid load on amyloid PET scans in cognitively normal and mildly impaired 1FL ADRC participants. The proposed research is of particular importance for the Hispanic-Latino population of South Florida, which is the largest and fastest growing ethnic minority in the US. Hispanics-Latinos have a disproportionately high burden of vascular risk factors and comorbidities, and they are largely underrepresented in AD research. Moreover, vascular comorbidities are modifiable and preventable. The results from the proposed investigations will uniquely position the team to start closing the gap in understanding of the mechanisms by which vascular phenotype contribute to AD pathology and to inform future strategies to reduce AD risk specifically tailored to high vascular and AD risk populations.

Follow On Funding: None at the time of reporting.

Collaboration: The investigators from the University of Miami (PI and other investigators) developed close relationships and collaborations with the investigators from the Mount Sinai's Wien Center for Alzheimer's Disease and Memory Disorders, University of Florida, and Florida International University, specifically with:

Mount Sinai's Wien Center for Alzheimer's Disease and Memory Disorders: Dr. Rundek (PI, University of Miami) together with Dr. Ranjan Duara and the 1Florida ADRC research team have supervised the overall image data quality assurance process.

University of Florida: Dr. David Loewenstein (University of Miami) together with Dr. Kevin Hanson (University of Florida) have supervised MRI data retrieval for this proposal to assure quality checks as recommended by the 1Florida ADRC consortium.

Florida International University: Dr. Mohammed Goryawala (University of Miami) and Dr. Malek Adjouadi (Florida International University) have performed MRI quality and data bias corrections. They were accompanied by two students from Dr. Adjouadi's laboratory at Florida International University learning the image quality checks and image bias corrections for educational and future research purposes.

Cross-Institutional Collaborations:

The clinical team of the VIP study that includes Dr. Rundek, PI, together with the clinical co-investigators Dr. David Loewenstein (University of Miami) and Dr. Ranjan Duara (Mount Sinai's Wien Center for Alzheimer's Disease and Memory Disorders) meets regularly to discuss the study progress, and potential issues and resolutions, to review analytical plan, and to plan future studies. This team meets every week at Mount Sinai (every Thursday from 4-6 pm).

The imaging team of the VIP study that includes Dr. Mohammed Goryawala (University of Miami), Dr. Malek Adjouadi (Florida International University) and Dr. David Loewenstein (University of Miami) meets regularly to discuss the MRI issues, review QC

pipelines, and assess progress of MRI reads and interpretation. This team meats bi-monthly at Florida International University (two Fridays per month from 3-5 pm)

Journals: None at the time of reporting.

Patents: None at the time of reporting.

25. **Grant #9AZ26:** Palliative Care Education in Assisted Living for Care Providers of Residents with Dementia

    Principal Investigator: Debra Dobbs, PhD

    Organization: University of South Florida

    Progress Report: Alzheimer's disease (AD) and other dementias are increasingly prevalent, with a current estimate of 5.7 million cases in the US. Florida has 540,000 individuals with AD. AD is the sixth leading cause of death in the US. There have been considerable efforts to improve end-of-life care for people with AD. In 1997, Medicare developed criteria for hospice eligibility for people with dementia (PWD), dramatically increasing access to hospice care for PWD. Now, 18% of the 1.4 million Americans who receive hospice care are PWD. PWD are increasingly cared for in Assisted Living (AL) and 40% die in these settings. A palliative care education in assisted living (PCEAL) for providers of PWD has been pilot-tested in 3 AL settings. The pilot work showed the PCEAL to be a feasible intervention. The scientific premise of this study is that PCEAL for nurses who provide dementia care in ALs in Florida will increase pain observations, improve documentation of advance care planning discussions for PWD and increase appropriate referrals to hospice. The current study is a two-year cluster randomized trial (CRT) among 12 AL facilities (k=12) and PWD (N=225) with a baseline, three, and six months post-intervention to examine the quality of care outcomes for PWD: 1) increased documentation of advance care planning discussions, 2) increased observations of pain, and 3) increased referral of hospice. A second study aim is to determine if improvements in outcomes from the PCEAL program are mediated through increased staff knowledge about palliative care.

    The expected health impact of the PCEAL program for nurses who care for persons with dementia in assisted living is to improve quality of end-of-life outcomes for persons with dementia who have serious illness specific to the study outcomes. A secondary health impact through the education and training is a decrease in avoidable hospitalizations. Positive care results of this research will promote the widespread use of the PCEAL program, an evidence-based, CEU-approved tool for educating nurses in assisted living to continue to improve the quality of end-of-life care for persons with dementia in assisted living settings.

    The research team in the first two quarters of the study enrolled eight assisted living sites, completed the four-week PCEAL intervention in two assisted living facilities, and began the PCEAL in one other treatment site. Research staff collected resident baseline

data in seven of the eight assisted living sites. Of 175 residents identified as eligible, 89 were enrolled. The study response rate for resident enrollment is 51%. Of 22 nurses identified as eligible, 16 licensed nurses were enrolled from eight assisted living sites to date. There are fewer nurses full-time, non-contract than anticipated in six of the eight ALs enrolled. In the treatment sites eight licensed nurses completed all of four PCEAL sessions and one nurse is in the process of completing the training sessions in the third treatment site. Data has been collected for pre- and post-intervention for nurse participants in four ALs and baseline for an additional three ALs. Nurses in the treatment ALs are conducting chart reviews for residents as designed in the protocols and conducting advance care planning discussions with family members. Pain observations have not been happening at a rate staff would expect from the treatment interventionist nurses. PC Champion two-month follow-ups have been conducted in two treatment ALs. Advanced Care Planning (ACP) discussions have been recorded and documented in a total of nine residents with dementias' family members in two treatment sites out of 20 residents in the study.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

26. **Grant #9AZ27:** A Pilot Study to Examine the Impact of an African Drumming for Dementia Program on African Americans with Mild Cognitive Impairment and Early Alzheimer's Disease and their Caregivers

Principal Investigator: Kyaien O Conner, PhD, LSW, MPH

Organization: University of South Florida

Progress Report: In Florida, approximately 12% of the senior population have a diagnosis of Alzheimer's disease (AD), and millions more are at risk for developing Alzheimer's disease and related disorders (ADRD) each year. Alzheimer's disease has detrimental effects on the functional quality of life of the individual living with AD and their caregivers. African Americans have a disproportionately high rate of AD, experience a high-rate of AD-related health disparities, are underrepresented in AD research, and are less likely to be evaluated and treated during early stages of the disease. Given concerns regarding detrimental side effects caused by antipsychotic drugs often used to treat negative psychological symptoms associated with AD, use of non-pharmacological care strategies have become vital. This highlights the urgent need to develop, implement and assess culturally relevant nonpharmacological interventions to help improve quality of life and psychosocial outcomes of African Americans living with AD and their caregivers.

Music interventions are low-cost interventions with benefits reported in previous studies including improvements on measures of anxiety, depression, agitation, mood, and autobiographical memory recall. Drumming as a music intervention is uniquely beneficial for individuals with dementia disorders. Drumming has been identified as an effective strategy for creating a communal experience among individuals living with ADRD. Individuals participate in variety of activities while drumming (e.g. rhythmic body movement, auditory stimulation, and chanting), which has been shown to yield multiple benefits for older adults with AD. African drumming may be particularly beneficial for African Americans living with AD. This culturally relevant and personalized approach to a music intervention has the potential to enhance the social environment for African Americans with AD and their caregivers, reduce caregiver burden, and improve mood and quality of life while simultaneously enhancing self-esteem and self-efficacy.

The current project supports the implementation and assessment of the feasibility and acceptability of an innovative African Drumming for Dementia intervention. Researchers will complete an open trial whereby staff will pilot test and determine the feasibility of the African Drumming for Dementia intervention for community-dwelling African Americans with early stage AD (N= 30) and their caregivers (N=30). The aims of this novel pilot project are to: 1.) assess the feasibility and acceptability of the African Drumming for Dementia intervention; 2.) assess psychosocial outcomes for persons living with early-stage AD including mood, quality of life, self-esteem, and self-efficacy; and 3.) assess the following psychosocial outcomes for caregivers: caregiver burden, mood, quality of life, self-esteem and self-efficacy. So far staff have recruited and begun the first intervention group with 16 participants (eight caregiver and participant dyads).

The project addresses focus areas 1.1, testing of non-pharmacologic interventions for behavioral expressions, and 1.2, interventions to reduce caregiver burden and enhance the social environment of individuals living with AD. Researchers expect results from this pilot to provide preliminary evidence that the African Drumming for Dementia intervention is a culturally meaningful therapeutic mechanism that can address measurable improvements in psychosocial outcomes for African Americans with AD and their caregivers.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

27. **Grant #9AZ28:** Visually-Assisted Mindful Music Listening Intervention for Persons Living with Dementia and their Caregivers: A Pilot Study

    Principal Investigator: Hongdao Meng, PhD, MPH

    Organization: University of South Florida

Progress Report: The research team has successfully recruited two study sites, enrolled 18 participants from the first site (45% of the targeted total enrollment of 40 participants), developed draft intervention protocols for the PLWD and FC groups, completed the first mindful music listening intervention for family caregivers (four individuals), and is currently in the middle of the second group of music intervention for persons living with dementia (nine individuals). The study team is adequately staffed with graduate and undergraduate research assistants and the College and Department have provided additional lab space for the project. Data collection for nine participants have been completed (pre-post surveys, actigraphy watch, and EMA measures), with data collection for nine additional participants expected in one month.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

28. **Grant #9AZ29:** Intracellular anti-Tau Proteins Engineered on a Hyperthermophilic Scaffold

    Principal Investigator: Jack M Webster, PhD

    Organization: University of South Florida

    Progress Report: Aggregation of the tau protein in neurons causes neurodegeneration. Tau aggregation correlates well with Alzheimer's disease progression, as well as other neurodegenerative diseases. The purpose of the project is to create engineered proteins that bind to the tau protein inside neuronal cells (were the aggregation begins) and inhibit the accumulation of neurotoxic aggregates.

    Project staff has created two engineered proteins using the Cold Shock Protein (CSP) scaffold that were rationally designed for the purpose of inhibiting tau aggregation. Assessment of these protein in an in vivo tau aggregation assay revealed that one of the two did reduce tau aggregation. These results also revealed that the parental non-engineered CSP delayed tau aggregation; however, this appears to be related to a natural tendency of the protein to bind to heparin (used to induce tau aggregation in the assay) rather than binding to tau. Therefore, researchers are evaluating mutant CSP variants for potential use as a modified, alternative scaffold that does not bind to heparin or DNA/RNA.

    Additionally, a library of double-stranded DNA fragments that contain degenerate codons at the desired positions for the randomized library designated CSP-Loop (CSP-L) have been created and inserted into a plasmid vector. Sequencing of eight individual clones suggests that 25% of products contain frame shift mutations relating to expected errors in oligonucleotide synthesis. The other 75% were correct variants with randomizations

in the engineered locations. First attempts at insertion of a library into the T7 phage vector were successful, however the titer and hence the library size was below target. Therefore project staff is working on optimization of library insert synthesis and purification in an attempt to increase the library size. Also, wild-type and rationally-designed CSP constructs were for the first time overexpressed in a human cell line. Some of the unusual characteristics of the CSP scaffold (which also make it a desirable protein engineering scaffold) presented a challenge in detection of cellular expression by standard western blotting techniques, but this technical challenge has been overcome in this reporting period. Confirmation of exogenous expression allows evaluation of CSP-variants in cell-based models of tau aggregation, for both rationally-designed constructs and those obtained from screening randomized libraries.

Successful development, characterization, and application of CSP constructs targeting tau will provide tools with which to study Alzheimer's disease as well as other tauopathies and may enable potential therapeutic protein strategies to reduce tau aggregation. Additionally, development of randomized libraries on this scaffold will enable screening and development of binders against targets other than tau.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

29. **Grant #9AZ30:** Impact of Adapted Dance on Mood and Physical Function among Alzheimer's Disease Assisted Living Residents

Principal Investigator: Crystal Bennett, PhD, RN

Organization: University of South Florida

Progress Report: Alzheimer's disease and related dementia disorders (ADRD) are the most common neurodegenerative disease in older adults and are the sixth leading cause of death in the United States. Almost 40% of assisted living residents have an ADRD diagnosis. Secondary symptoms of ADRD that are challenging to manage in the assisted living facility (ALF) and contribute to significant caregiver burden are agitation and declines in physical function. These symptoms lead to greater dependence on ALF staff and caregivers for assistance with activities of daily living. This is a pilot research project that will address Focus Area 1.1 (Behavioral) and will assess whether adapted dance improves psychological and physical secondary symptoms of ADRD in Northwest Floridians.

Dance is a promising intervention that can improve mood, physical function, and quality of life in older adults, including those with neurological conditions. However, adapted dance is not offered to those with ADRD in Northwest Florida ALF communities.

Whether adapted dance can improve agitation in those with ADRD residing in the ALF is not clear. Addressing this knowledge gap could support the use of adapted dance to improve quality of life in the ADRD population, specifically in Northwest Florida.

This project innovates by using a creative non-pharmacologic intervention to target secondary symptoms of ADRD. The aims for this project are as follows: 1a) assess the extent to which 12 weeks of adapted dance improves agitation; 1b) assess the extent to which 12 weeks of adapted dance improves balance, gait, and lower extremity function; and 1c) assess the extent to which 12 weeks of adapted dance decreases caregiver burden. An experimental design will be used with ADRD residents and their caregivers. ADRD residents will complete 12 weeks of adapted dance. Measures will be collected for agitation, balance, gait, lower extremity function, and caregiver burden at baseline and every four weeks for a total of 24 weeks. Participants will be randomly assigned to the adapted dance group or social stimulation control group. The control group will be participating in socially stimulating non-physical activities. At conclusion of the 12 weeks, the control group will then participate in the dance intervention for the same time period. Recruitment will take place in Northwest Florida ALFs. Residents will be screened for study participation using the Montreal Cognitive Assessment to assess cognitive ability, the Cohen-Mansfield Agitation Index to assess agitation, and the Timed-up-and-go test to assess mobility. Testing and the intervention will take place in Northwest Florida ALFs.

The 12-week adapted dance intervention will be a low-impact dance routine in which one foot is always in contact with the floor. Adapted dance can be modified specifically for those with cognitive and mobility limitations. An experienced dance instructor with over 25 years of experience will provide the dance instruction.

Measurements of outcomes will include using the Cohen-Mansfield Agitation Index and the Neuropsychiatric Inventory-Clinician Rating Scale to assess agitation; the Short Physical Performance Battery to assess balance, gait, and lower extremity function; and the Zarit Burden Interview to assess caregiver burden.

Follow On Funding: None at the time of reporting.

Collaboration: The University of West Florida in Pensacola, FL is the institution involved in the project. Dr. Crystal G. Bennett is the Principal Investigator and a faculty member with the Usha Kundu MD College of Health and the UWF School of Nursing. Dr. Rodney Guttmann is the co-investigator and a faculty member with the Hal Marcus College of Science and Engineering and Department of Biology. Dr. Raid Amin serves as project statistician and is a faculty member with the Hal Marcus College of Science and Engineering and Department of Mathematics & Statistics. Camila Medina-Pacheco is a UWF student in the Hal Marcus College of Science and Engineering, and Emily Bailey and Patricia Sirgo-Wirth are UWF nursing students in the Usha Kundu MD College of Health and School of Nursing. These three undergraduate students have received training and are performing research activities with project.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

30. **Grant #9AZ31:** Investigation of Alzheimer's Disease-Induced Circadian Dysfunction on Tau Production and Phosphorylation

    Principal Investigator: Joshua Gamsby

    Organization: University of South Florida

    Progress Report: Over the past quarter, research staff have made solid progress on the aims of the project. First, staff have ordered a mouse line from Jackson labs that expresses a particular type of pathology found in Alzheimer's disease, known as tauopathy, and initiated a breeding program to produce enough mice to eventually achieve aims 1-3. Secondly, staff have completed the production of a novel DNA construct that will allow staff to determine if the molecular circadian clock influences tau production. Staff have initiated testing this construct and plan to have completed this testing by the next quarterly report. Finally, staff have recently published some of the collaborative findings (listed below) and cited the FLDOH award(s) in the acknowledgement section.

    Follow On Funding: None at the time of reporting.

    Collaboration: None at the time of reporting.

    Journals: *Inhibition of casein kinase 1δ/ε improves cognitive-affective behavior and reduces amyloid load in the APP-PS1 mouse model of Alzheimer's disease.* Sundaram S, Nagaraj S, Mahoney H, Portugues A, Li W, Millsaps K, Faulkner J, Yunus A, Burns C, Bloom C, Said M, Pinto L, Azam S, Flores M, Henriksen A, Gamsby J, Gulick D. Sci Rep. 2019 Sep 24;9(1):13743. doi: 10.1038/s41598-019-50197-x

    Patents: None at the time of reporting.

APPENDIX C
FISCAL YEAR 2019-2020 ACTIVE GRANTS
(Funding Year 2017-2018)

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
8AZ02	Florida Atlantic University	Modi, Jigar	$ 100,000	$ 80,000	$ 20,000	03/19/2018	02/29/2020	No	No	No
8AZ03	Florida Institute of Technology	Liao, Yi	$ 100,000	$ 81,250	$ 18,750	02/09/2018	02/28/2020	Yes	Yes	Yes
8AZ04	Florida International University	Nair, Madhavan	$ 224,643	$ 88,920	$ 135,723	02/28/2018	02/28/2022	No	Yes	No
8AZ05	Florida State University	Carretta, Henry	$ 100,000	$ 77,916	$ 22,084	02/01/2018	02/28/2020	No	No	No
8AZ06	Mayo Clinic Jacksonville	Murray, Melissa Erin	$ 221,000	$ 116,641	$ 104,359	02/12/2018	02/28/2021	No	Yes	No
8AZ07	Mayo Clinic Jacksonville	Liu, Chia-Chen	$ 221,000	$ 114,799.30	$ 106,200.70	02/14/2018	02/28/2021	No	No	No
8AZ08	Mayo Clinic Jacksonville	Lucas, John A.	$ 200,000	$ 105,322.70	$ 94,677.30	02/08/2018	02/28/2021	No	No	Yes
8AZ11	Mount Sinai Medical Center	Duara, Ranjan	$ 96,643	$ 68,458	$ 28,185	05/21/2018	04/30/2020	No	No	No
8AZ12	University of Central Florida	Teter, Kenneth	$ 200,000	$ 75,006	$ 124,994	04/09/2018	02/28/2022	No	Yes	No
8AZ13	University of Central Florida	Hernandez, Florencio	$ 200,000	$ 158,327	$ 41,673	03/05/2018	02/28/2020	No	Yes	No
8AZ14	University of Central Florida	Sikorska-Simmons, Elzbieta	$ 95,784	$ 75,829	$ 19,955	03/05/2018	02/28/2020	No	No	No
8AZ15	University of Florida	Kesavalu, Lakshmyya	$ 221,000	$ 147,328	$ 73,672	02/21/2018	02/28/2020	No	No	No
8AZ16	University of Florida	Chakrabarty, Paramita	$ 221,000	$ 73,664	$ 147,336	02/08/2018	02/28/2022	No	No	No
8AZ17	University of Florida	Cottler, Linda B.	$ 200,000	$ 105,461.65	$ 94,538.35	03/05/2018	02/28/2021	No	Yes	No
8AZ18	University of Florida	Yachnis, Anthony T.	$ 99,987	$ 79,154	$ 20,833	01/25/2018	02/28/2020	No	No	No

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
8AZ19	University of Florida	Streit, Wolfgang J.	$ 96,643	$ 76,513	$ 20,130	02/01/2018	02/28/2020	No	Yes	No
8AZ20	University of Florida	Xu, Guilian	$ 99,577	$ 78,831	$ 20,746	02/21/2018	02/28/2020	No	No	No
8AZ22	University of Miami	Alperin, Noam	$ 221,000	$ 174,952	$ 46,048	02/16/2018	02/28/2020	No	Yes	Yes
8AZ23	University of Miami	Loewenstein, David	$ 450,844	$ 356,915	$ 93,929	02/14/2018	02/28/2020	No	No	Yes
8AZ24	University of Miami	Toborek, Michal	$ 221,000	$ 73,664	$ 147,336	02/07/2018	02/28/2022	No	No	No
8AZ25	University of Miami	Griswold, Anthony	$ 100,000	$ 83,330	$ 16,670	02/27/2018	08/31/2019	No	No	No
8AZ26	University of Miami	Dykxhoorn, Derek	$ 200,000	$ 133,328	$ 66,672	02/14/2018	02/28/2020	No	No	No
8AZ27	University of South Florida	Selenica, Maj-Linda B.	$ 100,000	$ 66,672	$ 33,328	02/09/2018	02/28/2020	No	No	No
8AZ28	University of South Florida	Nash, Kevin	$ 100,000	$ 66,672	$ 33,328	02/08/2018	02/28/2020	No	No	No
8AZ29	University of South Florida	Kang, David E.	$ 221,000	$ 174,559	$ 46,441	02/06/2018	02/28/2020	No	Yes	No
8AZ30	University of South Florida	Lee, Daniel C.	$ 200,000	$ 66,667	$ 133,333	02/23/2018	02/28/2022	Yes	No	No
8AZ32	The Roskamp Institute, Inc.	Keegan, Andrew	$ 99,576	$ 78,831	$ 20,745	03/02/2018	02/28/2020	No	No	No

ACTIVE RESEARCH GRANTS FISCAL YEAR 2019-2020
(Funding Year 2017-2018)

1. **Grant #8AZ02:** Neuroprotection of GCSF Gene Therapy in Alzheimer's Disease

   Principal Investigator: Jigar Modi, MD, PhD

   Organization: Florida Atlantic University

   Progress Report: The purpose of the project, for the period from October 1, 2018 to September 30, 2019, is to determine which hGCSF gene vectors designed provides the best results in animal-based [5 months old] Triple Transgenic Alzheimer's disease (3xTg-AD) models based on the molecular and cellular biomarkers and functional/behavioral tests. The biomarkers used for cell-protection and cell survivals include Bcl-2, p-Akt/Akt and OPA1, and those used for cell stress/injury and cell death include CHOP, GRP78, DRP1, Bax, and Beclin 1. The markers used for glutamate toxicity include NMDAR1, GlutR1, and GFAP.

   The highlights of the progress made during this period are summarized as follows:

   (1) Delivery of AAV-hGCSF gene vectors to mice of 3xTg-AD via eye drop method and demonstration of expression of hGCSF protein in the brain. The presence of hGCSF mRNA in the brain after infection with AAV-CMV-hGCSF, AAV-SYN-hGCSF, and AAV-SYN-HRE-hGCSF gene therapy was confirmed using real time PCR, qRT-PCR. And the expression of human GCSF protein (hGCSF) was confirmed by immunoblotting test.

   (2) Demonstration of effect of AAV-hGCSF gene on apoptosis markers like Bax (pro-apoptosis biomarker), along with cell survival marker Bcl2 (antiapoptosis biomarker) and P-Akt/Akt; reduction of gliosis e.g. GFAP (glial fibrillary acidic protein), and glutamate toxicity e.g. GlutR1 and NMDAR1; inflammation markers e.g. IL-6 and CD5 in mouse of 3xTg-AD model.

   (3) Demonstration of the efficacy of the delivered hGCSF gene therapy by showing a reduction of stress markers for endoplasmic reticulum (ER), e.g., GRP78, CHOP, ASK1 (apoptosis signal-regulating kinase 1) [upstream IRE1α pathway marker], ATF4 (activating transcription factor 4), [downstream PERK pathway marker] and Caspase-12, for autophagy marker, e.g., Beclin-1 and for mitochondrial marker, DRP1 and enhancer for mitochondrial functions e.g., OPA1. The locomotor activity was found to be significantly improved by hGCSF gene therapy with AAV-CMV-hGCSF and AAV-HRE-SYN-hGCSF. This supports the hypothesis that the presence of the hypoxia-response promoter (i.e., HRE) is critical for AD gene therapy.

   (4) Elucidation of the mechanism of AAV-hGCSF gene therapy in AD model including both neuro-protection and neuro-regeneration. The neuro-protective mechanism is supported by a reduction of cell stress markers and an increase of pro-cell survival markers as stated above in 2 and 3. The neuro regeneration mechanism is supported by

the observations that an increase of ChAT (Choline Acetyl Transferase), a marker of Cholinergic neurons along with increase of VAChT (Vesicular acetylcholine transporter), a specific marker to identify cholinergic synapses, and decrease of β-Amyloid (Aβ) protein, a marker of neurodegeneration was obtained using qRT-PCR and immunoblotting test after hGCSF gene therapy. The increased level of ChAT and decrease level of Aβ in 3xTg-AD mice model after administration of hGCSF gene therapy suggesting that therapy further delays the process of neural degeneration in the AD model.

(5) Clinicians reported that AD's patients had significantly lower CD34 (+) [marker for hematopoietic stem/ progenitor cells], and EPCs (CD34 positive endothelial progenitor cells) than the control subjects. The expression level of CD34 was increased by the administration of AAV-hGCSF gene therapy in 3xTg-AD model. This suggests that gene expression of hGCSF tends to increase CD34 induced neurogenesis response.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

2. **Grant #8AZ03:** CO Releasing Polymer Nanoparticles for Treatment of Alzheimer's Disease

   Principal Investigator: Yi Liao, PhD

   Organization: Florida Institute of Technology

   Progress Report: A new organic photo carbon monoxide releasing molecule (photoCORM) DK4, which showed superior properties compared to the same type of photoCORMs, has been developed and encapsulated in poly (butyl cyanoacrylate) (PBCA) nanoparticle. PBCA is a well-studied brain delivery polymer. The PBCA/DK4 nanoparticle showed good photoactivity and low cytotoxicity, and thus is a promising material for delivering CO to brain and studying the biomedical effects of CO. An article about this work was published in *Photochemistry and Photobiology Science* as the front cover of the issue.

   Previous studies have shown that ultrasound can open the blood-brain barrier and thus allow drugs to be delivered to brain. Therefore, ultrasound-responsive nanoparticles containing CORMs have been studied. One of the CORMs used was CORM2, which releases CO in the presence of some amino acids and proteins. CORM2 has shown many therapeutic effects including anti-inflammation and neuro-protecting effects in previous studies. Nanoparticles of pluronic, a FDA proved drug delivery polymer, and CORM2 were prepared and studied. Results showed that in the presence of cysteine, the amount of CO released under ultrasound is about twice of that without ultrasound.

This is a breakthrough since no ultrasound-responsive CO releasing material has been reported before. Researchers have filed an invention disclosure form for this discovery.

A new boron carboxylate CORM (nicotinyl alcohol) H2BCOOH has been synthesized and studied. NMR study showed that it was stable at room temperature and only slightly hydrolyzed (which led to CO release) over days. In the presence of H2O2, which is a ROS, the reaction was much faster and 25% of the CORM was hydrolyzed after two days. Since ROS is known to be related with AD, this type of CORM could be used to release CO more selectively to the locations related to AD. However, the reaction rate is still slow and must be improved.

Recent studies showed that the Myoglobin test, which is commonly used for measuring the CO released from CORMs, is not reliable in many cases since the sodium dithionite used in the test reacts with many CORMs. Staff have adapted a new method based on literature to measure the CO release. This method depends on the equilibrium between the CO in the solution and in the gas phase. Staff have used this method in the recent studies.

Follow On Funding: Community Foundation of Brevard - $26,000 (funded); National Institute of Health – $253,522 (pending).

Collaborations: *The project is conducted by collaboration between the PI, Yi Liao and co-PI Rudolf Wehmschulte. PhD students Hussam Alhamza, Osamah Alghazwat, and Adnan Elgattar and research associate Somayeh TalebzadehFarooji have been working on the project. Staff also collaborated with Dr. Bashur's group to study the biological effects of CORMs prepared in this project on AD. In addition, Dr. Kanekiyo at Mayo Clinic has been interested in using the CORM technique and collaborated with us on an NIH grant application.*

Journals: Adnan Elgattar, Kenyatta S. Washington, Somayeh Talebzadeh, Almutasim Alwagdani, Thaaer Khalil, Osamah Alghazwat, Sultan Alshammri, Hemant Pal, Chris Bashur and Yi Liao, Poly(butyl cyanoacrylate) nanoparticle containing an organic photoCORM, Photochemical & Photobiological Sciences, 2019, DOI: 10.1039/C9PP00287A. (This article has been chosen as the front cover of the coming issue.)

Patents: An invention disclosure about the ultrasound-responsive CORM nanoparticle has been submitted to FIT.

3. **Grant #8AZ04:** Therapeutic Role of Withaferin A and CRID3 in the Prevention of AD. A Novel Nanotechnology Approach

   Principal Investigator: Madhavan Nair, PhD

   Organization: Florida International University

   Progress Report: In this study, researchers have proposed to study the therapeutic efficacy of magneto-electric nanoparticle (MENP)-bound Withaferin A (WA), Cytokine

release inhibitory drug 3 (CRID3), in inhibiting amyloid beta (Aβ) production and associated NF-kB and NLRP3 inflammasome-mediated neuroinflammation in Alzheimer's disease (AD) both in vitro and in vivo models. To date, staff have observed that WA significantly inhibits the Aβ-levels in Aβ-overexpressing neuronal cells (SH-APP) in a dose dependent manner. Furthermore, staff found that WA inhibits the NF-kB mediated neuroinflammation in the mixed cell culture model (SH-APP cells along with microglia). Furthermore, to understand the mechanism of action of WA, staff studied the molecular mechanism of interaction of WA with Aβ protein by in-silico molecular dynamics simulations. The results demonstrated that WA binds to the middle region of Aβ protein and that the amino acid motif involved were FAEDVGS highlighting the mid-region of Aβ capture by WA. Three hydrogen bonds were formed between WA and the amino acids, ASN17, GLY15 and SER16. In addition, staff have observed that CRID3 significantly inhibits the Caspase 1 gene expression and concurrently the release of mature IL-1beta. These observations confirmed the therapeutic efficacy of CRID3 in inhibiting NLRP3-mediated neuroinflammation. Further, to investigate the comorbidity of HIV and AD in the genesis of Aβ production staff have observed that HIV-1 Tau protein induces Aβ production and WA inhibits Aβ production and improves the neuronal plasticity. Earlier studies suggest that Histone deacetylase 2 (HDAC2)-mediated epigenetic mechanisms play a major role in the genesis and neuropathology of AD. Researchers have observed that Mithramycin A (MTM), an HDAC2 inhibitor, significantly upregulated the synaptic plasticity gene expression and downregulated HDAC2 in SH-SY5Y cells overexpressing amyloid precursor protein (SH-APP cells). Therefore, the introduction of these agents targeting Aβ production, NLRP3-mediated neuroinflammation and HDAC2 levels will have a translational significance in the prevention of neuroinflammation and associated neurodegeneration in AD patients. Further, to address the issue of drug delivery and drug bioavailability in the CNS, staff have developed the WA/CRID3 loaded liposomal nano-formulations, and characterized and studied its cytotoxicity, BBB transmigration, and efficacy using the 3-dimensional BBB model. Currently, staff are developing and studying the complete nano-formulation containing MENP-bound CRID3 and encapsulation within the liposomes along with the WA. Simultaneously, staff are planning to examine the nano-formulations therapeutic efficacy in Alzheimer's disease (APP/PS1) mice model.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Sneham Tiwari, Venkata Atluri, Ajeet Kaushik, Adriana Yndart, Madhavan Nair. 2019. Alzheimer's disease: Pathogenesis, Diagnostics and Therapeutics. International Journal of Nanomedicine. 2019 (14), 5541-5554. Doi: 10.2147/IJN.S200490

Venkata Subba Rao Atluri, Sneham Tiwari, Melisa Rodriguez, Ajeet Kaushik, Adriana Yndart, Nagesh Kolishetti, Madhavan Nair. Inhibition of Aβ production, associated neuroinflammation and HDAC2 mediated epigenetic modifications prevent neuropathology in Alzheimer's disease. Under Communication.

Patents: None at the time of reporting.

4. **Grant #8AZ05:** Disparities in Health Services Utilization Across Racial/Ethnic Groups Among Persons with Alzheimer's Disease and Related Conditions

   Principal Investigator: Henry Carretta, PhD, MPH

   Organization: Florida State University

   Progress Report: The purpose of this study is to understand the association of individual characteristics of people on the observed differences in health outcome measures for racial minorities as compared with the Caucasian population among persons with Alzheimer's disease and Related Conditions (ADRC). Examples of Individual characteristics include: a person's age, education level, sex, the number and type of co-occurring health conditions, use of medical services and use of harmful substances like tobacco, alcohol, or drugs. An additional purpose of the study is to understand the relationship between where a person lives (in this case Florida counties) and observed differences in ADRC health outcome measures between racial minorities and Caucasians. Studying individual experiences in the context of where an individual lives has been demonstrated to provide a more complete understanding of differences in health outcomes. Factors outside of an individual that can impact a person's quality of life, health, and opportunities for improved life opportunities are sometimes referred to as the Social Determinants of Health (SDOH).

   It has been observed that individuals who might be considered disadvantaged because of where they live or because of their personal circumstance, are sometimes observed to achieve better health outcomes than would be expected based on those personal and residential circumstances. The African American (AA) population on average have fewer years of formal education, earn less money, and live in poorer neighborhoods as compared with advantaged white populations, and more often carry a higher burden of disease from ADRC. Yet in spite of those burdens, some AA individuals and populations appear able to overcome the disadvantages and life experiences and have health outcomes that are as good as or better than some advantaged populations. The unexpected outcomes in these AA populations are believed to be related to the level of resilience among some individuals and communities. This study's aim is therefore to identify Florida counties that are achieving better ADRC health outcomes than expected based on the characteristics of the individuals and the Florida county in which they reside.

   Preliminary estimates of the ADRC study population for 2010 reveal that 83% are white, 9% are African-American, and 8 percent are Hispanic. Sixty-five percent are female and the average age is 82 years. Certain chronic conditions are quite common in the ADRC study population. For example, 31% have type 2 diabetes, 30% have asthma or COPD, 78% have hypertension, and 42% have high cholesterol or other lipids. Persons with ADRC were nearly nine times greater odds of a hypertension diagnosis and 3.4 times greater odds of a diabetes diagnosis than persons without an ADRC diagnosis. Odds of

dying during 2010 were six times higher than the no ADRC population. About 12% of the ADRC population were eligible for Medicare before turning 65 due to a disability determination.

Follow On Funding: None at the time of reporting.

Collaboration: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

5. **Grant #8AZ06:** Quantitative Neuropathology and Biochemistry of Survival Differences in Hispanic Americans with Alzheimer's Disease

   Principal Investigator: Melissa Erin Murray, PhD

   Organization: Mayo Clinic Jacksonville

   Progress Report: In the next few decades, the United States population will become proportionally older and more ethnoracially diverse, contributing to a projected increase in the prevalence of dementia. By 2030, approximately one in five Americans will be over the age of 65 and by 2060, Hispanic Americans and black/African Americans are projected to constitute 29% and 14% of the population, respectively. The prevalence of dementia is estimated to more than double by 2050. Despite these trends, the understanding of dementia across ethnoracial groups remains limited and represents an important topic of investigation. The extent to which neuropathologic (*i.e.*, brain disease changes) and genetic factors contribute to disparities in cognitive deficits among ethnoracial groups remains poorly understood. Thus, clinical, genetic, and neuropathologic differences were investigated in Alzheimer's disease across three ethnoracial groups from the Florida Autopsied Multi-Ethnic (FLAME) study. Floridians who are seen by participating Memory Disorder Clinics throughout the state of Florida can register for autopsy regardless of sex, race, or ethnicity. All individuals evaluated in this grant have come to autopsy and are thus referred to as decedents.

   In the past year, the vascular disease score was completed for 1485 individuals, which included 73 Hispanic decedents, 18 black decedents, and 1391 white decedents. The vascular disease data was abstracted from neuropathology reports relevant to the cortex, basal ganglia, and hippocampus. This enabled the investigation of whether severity of vascular disease differs across ethnoracial groups. Toward the effort of appropriate matching, co-existing neuropathology (e.g. amygdala predominant Lewy bodies, Lewy body disease, and hippocampal sclerosis) was characterized. This required extensive review of available neuropathologic records and reassessment of some of the cases. Proportionally, more Hispanic and black decedents were identified to have greater co-existing pathology. Significant differences were not found in the admixture of co-existing neuropathology in Hispanic and black decedents. However, co-existing neuropathologies are more common in Hispanic and black decedents.

Family history review was also completed, as this may be an important factor to consider when evaluating survival. In order to assess family history, a family history of cognitive problems was recorded if noted in the clinical history. Information on individuals whose primary relative (mother, father, sibling) or secondary relative (grandparent, aunt, uncle, cousin) was identified to have cognitive problems was separately recorded. Proportionally Hispanic decedents may have a higher frequency of family members with cognitive problems, whereas black decedents may have a lower frequency.

Differences in regional distribution of neurofibrillary tangles across ethnoracial groups were quantitatively evaluated. Hispanic decedents were more severely affected in nearly all of the brain regions as compared to black decedents and white decedents in the FLAME cohort. Interestingly, the black decedents were more severely affected in the limbic regions, but not cortical regions compared to white decedents. The nucleus basalis of Meynert (nbM) is the cholinergic hub, which sends cholinergic projections throughout the cortex. It is worth noting that the nbM of Hispanic decedents had more tangles than white decedents, but black decedents had fewer.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Liesinger A.M., Graff-Radford N.R., Duara R., Aziz A., Hinkle K.M., DiLello S.K., Johnson M.F., Ertekin-Taner N., Ross O.A., Dickson D.W., **Murray M.E.*** (2018) *Sex and age interact to determine clinicopathologic differences in Alzheimer's disease.* Acta Neuropatholo. 136(6):873-885. PMID: 30219939 – Published December 2018

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neuropathologic subtypes of Alzheimer's disease. JAMA Neurology (Epub ahead of print).

Patents: None at the time of reporting.

6. **Grant #8AZ07:** Impact of TREM2 Variants on Microglial Function and Alzheimer's Disease Pathology

   Principal Investigator: Chia-Chen Liu, PhD

   Organization: Mayo Clinic Jacksonville

   Progress Report: The aging population in the United States will lead to an alarming number of Alzheimer's disease (AD) cases with no clear disease-modifying treatment at hand. In addition to the presence of protein aggregates, including senile plaques composed of amyloid-β (Aβ) and neurofibrillary tangles (NFT), one important feature of AD is neuroinflammation associated with regions of pathology. Microglia are the innate immune cells of the brain that are posited to be the first sensors of local damage. Recent discovery of several AD risk genes, such as triggering receptor expressed on myeloid cells 2 (TREM2) has been shown to influence microglial functions, highlighting the role of this cell type in AD development. Genetic studies showed that a rare R47H variant (Arg-47-His) significantly increases AD risk to a similar degree compared with having apolipoprotein E (*APOE4*). TREM2 is an innate immune receptor primarily expressed by microglia in the brain and is involved in immune defense in the brain. For example, it plays a critical role in neuroinflammatory responses, clearing out damaged neuronal debris. Previous findings indicate that loss of TREM2 functions leads to an increase in Aβ accumulation and neuronal loss in AD mouse models. However, it remains unclear how this AD risk variant (TREM2-R47H) affects the functions of microglia and amyloid plaque development.

   Research staff have developed a novel mouse model to express TREM2 wild-type (WT) or TREM2-R47H to be present only in microglia. The inducible feature of the model allows staff to control when and where the TREM2 will be expressed. Using this model, staff will address how this AD-associated risk variant altered the behaviors and functions of microglia which may contribute to AD disease development. In addition, staff will examine whether and how this TREM2 variant compromises the function of microglia for their ability to remove or clear the toxic protein aggregates, such as Aβ. Research staff expect that this AD-associated mutation, TREM2-R47H, will cause microglia dysfunction, enhance neuroinflammation and accelerate the development of AD. Researchers have successfully generated the animal models expressing human TREM2 WT or R47H mutant in this fiscal period. Upon induction, staff showed that TREM2 is only expressed in microglia, the major resident immune cells in the brain. In addition, staff examined hTREM2, and specific microglia-related genes (such as Tyrobp, Aif1, Hexb, C1qa, C1qb, Csf1r, cd33 and cd68, etc.) by real-time PCR. Researchers found that hTREM2 expression was upregulated in both TREM2-WT or TREM2-R47H mice, further confirming the expression of TREM2 and the variant in the mouse models. More

importantly, staff found that microglia from TREM2-R47H mice had less branches and junctions of the processes compared with TREM2 WT microglia. Such differences in morphology were previously shown to reflect the differential activation status, indicating that microglia carrying TREM2-R47H variant may exhibit loss-of-function phenotype compared with TREM2-WT microglia. Staff also recently performed RNA-sequencing on the brain of these experimental mice. Interestingly, networks related to circadian regulation was abnormally up-regulated in mice expressing TREM2-R47H. Staff will further validate this pathway and determine the potential mechanisms underlying the microglial dysfunctions. In addition, staff have bred this mouse model with amyloid model mice and are currently expanding the mouse colony. Once the experimental cohort is established, staff will explore whether microglia carrying AD-risk have impaired functions in the regulation of amyloid development. Understanding how microglia function and their inflammatory responses in the context of AD may facilitate the development of novel therapeutic strategies to treat AD.

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 #8AZ08:** Evaluating the Impact of a Dementia-Caring Community Model on African Americans with Alzheimer's Disease and their Care Partners.

   Principal Investigator: John A. Lucas, PhD

   Organization: Mayo Clinic Jacksonville

   Progress Report: Ethnic minority communities often experience a disproportionate degree of health disparities related to Alzheimer’s disease (AD) and dementia. African Americans in particular have a significantly higher prevalence of AD than Caucasians, but typically do not seek evaluation until much later in the disease course. A number of sociocultural factors contribute to this disparity, including lack of dementia education, mistrust of the medical establishment, and limited knowledge of available resources. Communities can play an important role in helping residents with AD and their care partners obtain appropriate services and overcome the challenges and stigma of dementia that threaten quality of life, social well-being, and functional independence. The National Alzheimer's Project Act (NAPA) provides a roadmap to help communities become more 'dementia-caring’, through the Dementia Friendly American (DFA) model. The primary aim of the current study is to assess the impact of the DFA model on dementia knowledge in an African American community. Additional aims seek to explore the impact of this model on beliefs about dementia and quality of life of African American caregivers of loved ones with dementia.

A total of 269 community-dwelling African Americans have participated in this study to date. Of these, 111 residents of Jacksonville's New Town Success Zone (NTSZ) and 78 residents of the Jacksonville urban core completed surveys evaluating dementia knowledge and caregiver burden before and after an informational presentation about Alzheimer's disease and dementia. Following the presentation, the NTSZ group engaged in an hour of focus group discussions about the impact of dementia on the community, whereas residents from the Jacksonville urban core engaged in a social hour, during which time dementia experts were on hand to address questions raised by participants. Approximately two months after the informational event, participants returned for a second visit, at which time dementia knowledge was re-assessed. In addition, a new group of 80 NTSZ residents completed the knowledge surveys while attending a community health event that was unrelated to dementia.

The main finding thus far is that knowledge of dementia improved immediately after the educational presentation in both conditions (focus group vs. social hour) but two months later, people who participated in DFA focus groups retained a significantly greater amount of that information than people who attended the social event.

The findings to date suggest that dementia education presented in an ethnic minority community is retained to a measurably better degree when the information is followed immediately by engagement in a scripted group discussion exercise about dementia, such as that provided in the NAPA DFA focus group materials.

Follow on Funding: Jacksonville Community Foundation $13,000 (pending)

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

8. **Grant #8AZ11:** Impact of the Modified MindSet Training Program on Maintaining Optimal Function Among Early Alzheimer's Patients and their Care Partners

   Principal Investigator: Sindy Goenaga, MD, MPH

   Organization: Mount Sinai Medical Center

   Progress Report: This is a novel adaptation of a program originally developed for cognitive training of persons with late mild cognitive impairment (MCI) and early Alzheimer's Disease (AD). This pilot project capitalizes on the strengths of this previous work and adds a component designed to help the care provider become a therapy extender, increase communication skills of the care recipient (CR) and care provider (CP), as well as to provide stress management and increased coping skills. Cognitive evaluations with a battery of neuropsychological and functional tests and measures of stress and anxiety are administered before and after the six-week program to determine

the impact of the program in every care recipient and care provider. A satisfaction survey is also conducted after the program is completed.

This is a unique and novel approach for dually treating both the CR and CP simultaneously which staff believe will have a synergistic effect in producing improvement for both members of the dyad. The CP will be a therapy extender by helping the CR with cognitive training homework, and the intervention will be administered to dyads in a small group setting.

If successful, this dual-target intervention will have substantial health impact in that it can enhance cognition and function in early AD patients and may reduce the rate of cognitive decline and reduce both CR and CP's burden. Since depression and burden can lead to adverse outcomes such as physical illness and institutionalization, this cost-effective non-pharmacological approach has a highly significant health implication.

This is a pilot study to examine the feasibility and acceptability of a novel intervention which provides simultaneous treatment to both the CR and the CP. An intention to treat (ITT) paradigm will be employed in a subsequent phase of this protocol, and additional funding for this protocol is currently being sought. The ITT paradigm includes every subject who is randomized according to a randomized treatment assignment, ignoring non-compliance, protocol deviations, withdrawal, and anything that happens after randomization.

The results of this important pilot study can provide critical information to design and refine a larger scale trial that provides a brief intervention to improve the life of both care recipients and care providers. Five training groups of CR and CP dyads have been completed. A total of 58 participants were recruited into this project. Of those recruited only 50 participant signed informed consents and underwent initial pre-testing. During the training sessions four participants dropped out of the study. Post-testing was completed in 46 participants. The following primary outcomes will be analyzed at the completion of the study: (1) change in cognitive measures and (2) change in quality of life measure.

Follow On Funding: None at the time of reporting.

Collaborations: University of Miami, Department of Psychiatry and Behavioral Sciences, Miami, FL (David A Loewenstein, PhD and Mathew Caplan, MHC)

Journals: None at the time of reporting.

Patents: None at the time of reporting.

9. **Grant #8AZ12:** Protein Disulfide Isomerase Uses Conditional Disorder as a Disaggregase Mechanism to Detoxify Amyloid Beta Fibrils

   Principal Investigator: Kenneth Teter, PhD

   Organization: University of Central Florida

Progress Report: Researchers propose that protein disulfide isomerase (PDI) can act as a "disaggregase" to dissolve and detoxify aggregated fibrils of the Aβ peptide. Thus, recombinant PDI could be used as a novel therapeutic agent for the clearance of extracellular Aβ fibrils that contribute to Alzheimer's disease. Staff will pursue this possibility by identifying the minimal PDI fragment with disaggregase activity and the molecular mechanism for its neuroprotective function.

PDI has an **abb'xa'** structural organization that consists of two thioredoxin-like catalytic domains (**a** & **a'**) separated by two non-catalytic domains (**b** & **b'**) and an **x** linker. Researchers predict the disaggregase activity of PDI is activated when substrate binding to the **b** domain transmits a signal through the **b'x** domains for unfolding of the **a'** domain. The expanded hydrodynamic size of the unfolded **a'** domain subsequently functions as a wedge to push against two or more peptides in the Aβ aggregate. This provides a mechanical force to break apart nascent aggregates of Aβ. In the context of this model, staff purified a panel of PDI deletion constructs to evaluate the structural stability of PDI and its ability to undergo rounds of unfolding and refolding. Biophysical studies indicated that, consistent with the disaggregase model, the **a'** domain of PDI is more prone to unfolding than the rest of the protein but can still refold after denaturation. Additional experiments with the deletion constructs suggest that the **a'** domain of PDI is necessary for its inhibition of Aβ fibrillization. Other studies with full-length PDI found that the inhibition of Aβ aggregation is completely effective at a 1:10 molar ratio of PDI:Aβ, partially effective at a 1:50 ratio, and ineffective at a 1:100 ratio. These observations provide new insight on the dynamic structural organization of PDI and its interaction with Aβ.

In the 2018-2019 reporting period, researchers published the structural study on the domain-specific unfolding/refolding of PDI in *Biochemistry*. This work was also presented in poster format at the national 2019 Experimental Biology meeting. Another poster on the contributions of individual PDI domains to its disaggregase activity was presented at both the 2019 Experimental Biology meeting and the St. Jude Children's Research Hospital National Symposium for Undergraduate Research. This project will be the main focus of work in the next reporting period.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Guyette J., Evangelista B., Tatulian S.A., and Teter K, Stability and Conformational Resilience of Protein Disulfide Isomerase, **Biochemistry**, 2019, 58(34):3572-3584. doi: 10.1021/acs.biochem.9b00405 PMID: 31393106

Patents: None at the time of reporting.

10. **Grant #8AZ13:** Optical Characterization of the Aggregation (Change in Size, Fibril Formation), Accompanying Structural Changes, and Membrane Pore Formation.

    Principal Investigator: Florencio Hernandez, PhD

Organization: University of Central Florida

Progress Report: The purpose of this project is to provide a clearer picture of the mechanism of development of Alzheimer's disease by correlating specific structural factors of Amyloid Beta with their toxicity.

Alzheimer's disease is the most common form of dementia in people 65 years of age and older, and accounts for 60-70% of dementia. According to the 2013 Alzheimer's disease Facts and Figures, an estimated 5 million people suffer from Alzheimer's disease in the United States and the number of cases is projected to triple by 2050. In the state of Florida there are more than 500,000 individuals with Alzheimer's disease, yet only 12% have been diagnosed. Alzheimer's disease is characterized by a progressive, irreversible deterioration of the patient's cognitive function. Many Alzheimer's patients, in the late stages of the disease, need 24/7 specialized care. Additionally, this disease is the sixth leading cause of death in the country and poses a large financial and social burden in families and society. Therefore, it has become a major public health concern and a research priority. Despite the tremendous basic and clinical research efforts, no effective therapies have been developed to treat Alzheimer's disease, mainly because the mechanism of development of the disease is still unresolved. By combining different expertise in optics, spectroscopy and biophysics, and computational chemistry, this research project promises to lead to a better understanding of the still-mysterious mechanism. To achieve this goal, the research project staff are trying to elucidate what the structural determinants of Amyloid Beta toxicity are and how these structural features correlate with membrane binding and permeabilization in Alzheimer's disease, as well as what are the driving forces and dynamics for plaque formation in the brain.

This period the research staff determined the structural conformation of large structures known to be present in AD and modeled the driven forces and dynamics that lead to formation of aggregates that can eventually form organized clusters. The team has proposed a new hypothesis for the formation of plaques in the brain. The research staff was also able to investigate the potential membrane pore formation capabilities of peptide fragments of seven synthetic fragments, and formation of ion channels with fast methods.

These novel results shed light on the potential mechanism and dynamics of development of the disease and may open an avenue to understand the possible implications of plaque formation and the effect of cholesterol on the development of Alzheimer's disease.

Follow On Funding: None at the time of reporting.

Collaborations: Five graduate students (Eduardo Romero, Christopher Felton, Nabin Kandel, Molla Manjurul Islam, Faisal Abedin) and three undergraduate students (Abdel Rahman Naser, Alea Sterling and Melanie Rodriguez) have been trained/mentored during the reporting period.

Journals: Kandel N., Matos J. O., Tatulian S. A. Structure of amyloid b25-35 in lipid environment and cholesterol- dependent membrane pore formation. *Scientific Reports* 2019 Feb 25;9(1):2689. doi: 10.1038/s41598- 019-38749-7.

Tatulian, S.A. and Kandel, N., Membrane Pore Formation by Peptides Studied by Fluorescence Techniques. *Methods Mol. Biol.* 2019, 2003, 449-464.

Eduardo E. Romero, Florencio E. Hernandez, "*Role of the Amyloid-b(1-42) Electric Dipole Moment on Fibrils Formation*", ACS Omega 2019, currently under review.

Patents: None at the time of reporting.

11. **Grant #8AZ14:** Factors Influencing Family Caregivers' Medical Decision-Making for Patients with Advanced Alzheimer's Disease

    Principal Investigator: Elzbieta Sikorska-Simmons, PhD

    Organization: University of Central Florida

    Progress Report: During this reporting period research staff 1) completed the first wave of interviews, 2) completed the second wave of interviews, 3) completed 90% of the third wave of interviews with family caregivers, 4) transcribed the completed interviews, and 5) started analyzing the qualitative data. Each interview was conducted in-person by the Principal Investigator and lasted on average 1-2 hours. The interviews were transcribed by the trained research assistant (a total of 1600 pages so far). The transcribed interviews contain rich qualitative data about caregivers' experiences with medical decision-making in the context of advanced dementia. The most common decisions were those related to seeking diagnostic services, routine medical care, medications, management of symptoms, nursing home placement, and hospice. Staff plan to finish the data collection (i.e., the third wave of interviews) by the end of October 2019, and will focus on the analysis and preparation of manuscripts during the remining stages of this project.

    In addition, a second grant-related paper was accepted for presentation at the ICGNHA 2020 International Conference on Geriatric Nursing for Healthy Aging ("Medical Decision-Making in Advanced Dementia from the Family Caregivers' Perspective: A Qualitative Study") June 25-26, 2020, Oslo, Norway. Staff are also preparing a manuscript based on this presentation.

    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 #8AZ15:** Periodontal Bacteria Augment Progression of Abeta; and Tau Pathology

Principal Investigator: Lakshmyya Kesavalu, BVSc., MS, SCC

Organization: University of Florida

Progress Report: Alzheimer's disease (AD) is marked by a progressive loss of memory. AD is a complex disease and researchers do not know the causes, with both environmental and genetic risk factors contributing to its onset. Several epidemiological, clinical, and molecular studies have shown that chronic gum disease in the mouth associated with gum swelling and redness (co-morbidity or cofactor) is linked with increased risk and progression of varying forms of memory loss, including AD. The purpose of this project is to determine the possible causal association between gum disease bacteria and AD. There are two bacteria [*Treponema denticola* (*T. denticola*) periodontal bacteria and *Streptococcus gordonii* (*S. gordonii*) non-periodontal bacteria] proposed for examination in augmenting progression of Amyloid β and tau pathology in TgCRND8 and nonTgCRND8 mice model of AD-like amyloidosis.

Numerous studies link gum disease-associated chronic inflammation with increased risk of dementia, including AD. Plasma levels of antibodies to gum disease bacteria are significantly higher in AD patients. One study directly showed the presence of seven different gum disease bacteria in Alzheimer's diseases patients' brains. Staff also observed oral bacteria component lipopolysaccharide (LPS) present in four out of 10 Alzheimer's disease brains. Researchers do not know the mechanism by which gum disease may be considered a risk factor for Alzheimer's disease.

The specific aims are to explore the gum disease bacteria *T. denticola* infection in regulating brain nerve damage in mouse model of AD-like amyloidosis. Specific Aim 1. Investigate the role of *T. denticola* in regulating of amyloid β peptide (plaque pathology in mouse model of AD-like amyloidosis. Staff conducted this study in two batches as the transgenic mice breeding yield is low (30-40%) and also sudden death syndrome is high (50-60%) in this mice strain. At the end of the study, staff will pool the mice data and analyze the data for statistical significance. SPECIFIC AIM 2: Determine whether oral spirochete *T. denticola* directly injected into the brain can seed amyloid deposition in TgCRND8 mouse model of AD-like amyloidosis. Research staff have completed Specific Aim 2 animal experiments. The sample analyses for both Aim 1 and Aim 2 studies are in progress. A pilot study was performed to enumerate the *T. denticola* from brain in APP-CRND8 mice after intracranial infection at 1, 4, 8, and 24 hour intervals. The colony PCR was performed to confirm the presence of *T. denticola*. DNA sequencing study is in progress. The Immunohistostaining analysis revealed that mice infected with *T. denticola* and *S. gordonii* induced higher Aβ plaques and GFAP expression in APP-TgCRND8 mice brain. Immunohistostaining for all other groups are in progress.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

13. **Grant #8AZ16:** Towards Understanding the Biological Role of Newly Discovered Alzheimer's Disease Susceptibility Genes Affecting Immune Function

    Principal Investigator: Paramita Chakrabarty, PhD

    Organization: University of Florida

    Progress Report: In Florida, 7031 patients died from Alzheimer's disease in 2015, making it the 6th leading cause of death in the state. More grim statistics show that there is a projected increase of ~33% in patient burden between 2015 and 2018 for AD (www.alz.org). Countrywide, the total value of unpaid care for AD patients has risen to $16 billion (2017) and the cost of caring for dementia patients (including AD) is now at a staggering $277 billion (2018). This cost is projected to increase to $1.1 trillion (in today's dollars) by mid-century. The need of the hour is to find cures as soon as possible. To do this, a basic cornerstone of research–creating and understanding disease-relevant models – has to be supported. Funding from the state of Florida has enabled this lab to create new mouse models that can help researchers understand how neuroinflammation affects disease progression in mouse models of amyloid β plaques, one of the earliest signs of AD dementia. These mouse models are unique and no other lab in the world has reported any similar data. These mouse models were created in this lab to understand the function of ABI3 and PLCG2, two newly-discovered AD risk genes that are critical components of the neuroimmune milieu in the brain. Given that recent research has uncovered a strong genetic association between innate immunity and AD, there is a strong scientific rationale for pursuing research on characterizing therapies targeting immune function in the brain as it relates to AD.

    Research staff have successfully created the mouse models and aged these to a point where they are equivalent to ~50-year-old humans. Staff have reported that (a) removing the Abi3 gene results in lowered Aβ plaque burden whereas (b) removing the Plcg2 gene alters microglial function but does not alter Aβ plaque burden.

    During this time, the project has led to at least one international platform presentation at the International AD/PD Conference (2019), several local presentations and AD-related lab training to various undergraduate mentees, rotating graduate students, and summer interns.

    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 #8AZ17:** Precision Public Health Approaches to Reduce Disparities in Memory Disorder Screening in Rural Minority Communities

    Principal Investigator: Linda B. Cottler, PhD, MPH, FACE

    Organization: University of Florida

    Progress Report: Only 1 in 10 older adults who has Alzheimer's disease (AD) receives a diagnosis. Additionally, 1 in 3 older adults who die have AD. Early identification of cases can reduce healthcare costs. It is critical to increase screening and identify AD cases, especially among rural residents and minorities. The aim of this project is to reduce health disparities for AD in mortality among older adults, 60 or older, in Florida by decreasing barriers to care. Target counties (Alachua, Putnam, Marion, Bay, Calhoun, Gulf, Jackson, Washington, and Wakulla) in the study include areas with the highest AD age-adjusted death rates; however, these areas have the lowest AD case rates, indicating the need for screening.

    A key reason for this lack of screening includes lack of knowledge and misperceptions by primary care physicians on the consequences of undiagnosed and untreated AD. A continuing medical education video titled, "From Brain Disorders to Brain Health: Prevention and Management of Cognitive Decline and Dementia" has been conceptualized and filmed. This training has been conceptualized for practitioners in target counties to enhance knowledge of cognitive impairment and dementia risk factors, screening and diagnosis, treatment, and available resources for patients and practitioners. Primary care physicians in target counties have been identified to contact for participation in the project.

    The Community Health Worker (CHW) model is used to screen older adults; CHWs recruit older adults in their communities. CHWs are actively working in Alachua, Putnam, Marion, Jackson, and Calhoun Counties, and recruitment is underway for CHWs in remaining counties. CHWs administer an intake to assess medical history as well as conduct an AD knowledge questionnaire and Montreal Cognitive Assessment (MoCA). Older adults who score less than 26 (highest score = 30) on the MoCA are referred to their primary care physician for further evaluation and possible referral to a memory disorder clinic. As of September 30, 2019, HealthStreet has enrolled a total of 2,474 older adults, and this project contributed 369 older adults to the Florida AD registry and HealthStreet membership. Among the 369 older adults enrolled in the project, 357 completed the AD questionnaire, and 238 completed the MoCA. One hundred fifty-three (153) older adults received a MoCA score less than 26, indicating the need for further assessment.

    Staff continue to build both the registry of Florida community-dwelling older adults who may be interested in research participation and the statewide infrastructure to link older adults to cognitive screening and related health research through CHW recruitment, networking and partnerships, and training for cognitive impairment screening in nine (9) Florida counties. The CHW model which includes information, education, connections to

local community and medical services, and follow-up at 60 and 120 days has demonstrated an increase in AD knowledge by the older adults. This is an important finding as it provides the basis for additional interventions to improve AD-related health literacy. This project is increasing AD screening among older adults and providing education to physicians in rural areas to improve the identification of cases.

Follow On Funding: None at the time of reporting.

Collaborations:

University of Florida, College of Public Health and Health Professions/College of Medicine, Department of Epidemiology, Gainesville FL: The Department of Epidemiology is responsible for the oversight and coordination of all aspects of the project. Dr. Linda Cottler serves as the principal investigator of the project and is currently the Associate Dean for Research for the College of Public Health and Health Professions and Dean's Professor in the Department of Epidemiology. Dr. Catherine Striley serves as a co-investigator on the project and is a Research Associate Professor in the Department of Epidemiology. Pre-doctoral student, Shawnta Lloyd, serves as the Project Coordinator for the project.

University of Florida, College of Medicine, Department of Neurology, Gainesville FL: Dr. Demetrius Maraganore is a co-investigator on this project and is the B.J. and Eve Wilder Professor in the Department of Neurology. Dr. Maraganore conceptualized and hosted the continuing medical educational training for primary care physicians as well as recruited the speakers to participate in training. In addition, Dr. Maraganore completed the administrative activities necessary for primary care physicians to receive credit for completing the continuing medical education.

University of Florida, College of Medicine, Continuing Medical Education, Gainesville FL: Project investigators are working with Edward Sheridan in the Office of Continuing Medical Education to make the CME available to clinicians.

UF Health Educational Technologies, Telehealth & Video Services: Project investigators worked with Zachary Vick (Telehealth & Video Services) and Amanda Tarantino (myTraining System Administrator) to record and edit the CME for clinicians.

University of Florida, HealthStreet, Gainesville FL: HealthStreet employs CHWs who go into the community to recruit and enroll community members into the project. CHWs meet community members where they live, work, and recreate to recruit potential members. CHWs are responsible for administering the HealthStreet intake which assesses medical history, medical concerns, and willingness to participate in research. In addition, CHWs administer the AD knowledge questionnaire and the MoCA to assess cognitive health in community members. Community members who receive a score of less than 26 on the MoCA are referred to their primary physician by the CHW.

HealthStreet provides training to CHWs. An online distance-learning platform has been developed to ensure high-quality training for the new CHWs and volunteers. This

distance-learning platform allows the program to reach all parts of the state and ensures the fidelity of the model. After the completion of online training, a senior CHW at UF HealthStreet and a faculty member in the Department of Epidemiology certify new CHWs and confirm their readiness to enter the field. As a final step in training, a senior CHW from HealthStreet travels to the county in which the CHW will recruit older community members. The new CHW has the opportunity to shadow the senior CHW and enhance their familiarity with the intake process. This method of shadowing is beneficial to the new CHW as it allows the CHW to observe and participate in the successful recruitment of older adults in their own communities.

Furthermore, HealthStreet works to build relationships with community partners to increase enrollment. Partnerships include Senior Nutrition Meal Sites, Senior Centers, Housing Authorities, Elder Care, and the faith community in Marion, Putnam, and Alachua Counties. These partnerships provide opportunities for greater reach into rural communities by using familiar locations and working with those who are trusted in the communities.

A resource is also available at the HealthStreet site for those who may benefit from it and who live in Alachua County. This is the College of Public Health and Health Professions Department of Clinical and Health Psychology faculty-supervised Neurocognitive Screening Initiative (NSI), which focuses on providing a cognitive assessment service to adults who have concerns about their thinking and/or memory. The NSI aims to offer feedback regarding cognitive strengths and weaknesses and brain health education. This activity is held monthly at the HealthStreet facility. This is not a substitution for referral to the Memory Disorder Clinics.

Alzheimer's Association: Staff are collaborating with Deann Marasco (Director, Health Systems, State of Florida, Alzheimer's Association) and Audrey Coachman (Program Manager, Alzheimer's Association, Central and North Florida Chapter) to increase screening and knowledge of dementia in underserved areas in the central and northwestern Florida areas.

Northwestern Florida (Bay, Gulf, Calhoun, Jackson, Washington, and Wakulla counties): Clinical and Translational Science Institute (CTSI)-affiliated faculty at Florida State University, WYBT AM 1000 Radio, The University of Florida's Institute of Food and Agricultural Sciences (UF/IFAS), Blountstown Library, Jackson County Libraries, Jackson County Senior Citizens Center, and Calhoun County Senior Citizens Center.

Marion County Partnerships: Seven Senior Nutrition meal sites: Belleview, Dunnellon, Flemington, Forest, Marion Café, Marion Oaks, Sparr, Barbara Gaskin Washington Adult Activity Center (for Seniors), Eighth Avenue Adult Activity Center (for Seniors), and Marion County Libraries.

Putnam County Partnerships: Food Pantries: Heart of Putnam Food Pantry, St. Andrews Food Pantry, Barry Manor Meal Site-senior meal site, First Baptist Church

Farmshare, First Presbyterian Church, Putnam Housing Authority, Putnam Christian Services, American Legion, Hitchcock's Markets, and Interlachen Soup Kitchen.

Student Training: A total of 18 undergraduate students and two graduate students received training through this project (Gender: Males-3, Females-17; Race: White-8, Hispanic-4, Black-4, Asian-4).

Journals: Serdarevic M, Gurka K, Striley CW, Leeman R. Cottler LB. Sex Differences in Prescription Opioid Use Patterns Assessed through a Community Engagement Program in Florida. Drug and Alcohol Dependence. (in press).

Patents: None at the time of reporting.

15. **Grant #8AZ18:** Investigations of Neuropathologies Targeted by Clinical Trials in Alzheimer's Disease Patients

    Principal Investigator: Anthony T. Yachnis, MD

    Organization: University of Florida

    Progress Report: Recruitment materials were generated for this project and received approval from the local IRB for distribution to clinical research sites. Additional clinical research sites were identified throughout the state of Florida. The research coordinator reached out to these locations to describe the efforts on brain banking. The research coordinator traveled to several of these sites, including Bradenton Research Clinic, Bradenton, FL; Restar Medical Research, Ocala, FL; and The Anchor Clinic in Pensacola, FL, within the past year. At each site, the research coordinator delivered a presentation describing the brain banking efforts and the value of individuals affected by Alzheimer's disease to participate in this effort. In addition, these recruitment materials have been distributed to additional clinical sites.

    Recruitment efforts are beginning to come to fruition; two individuals that participated in clinical trials came to autopsy within the past few months. Autopsies and neuropathological analysis are in progress.

    Follow on Funding: None at the time of reporting.

    Collaborations: None at the time of reporting.

    Journals: None at the time of reporting.

    Patents: None at the time of reporting.

16. **Grant #8AZ19:** Role of Microglia in Primary Age Related Tauopathy and in Sporadic (Late-Onset) Alzheimer's Disease

    Principal Investigator: Wolfgang J. Streit, PhD

    Organization: University of Florida

Progress Report: Work on this project has been progressing as proposed originally, i.e. human brain samples have been studied in detail with regard to brain microglial cells and tau pathology. The vast majority of tissue samples were obtained from individuals who at the time of their deaths had no symptoms of cognitive impairment or dementia in their medical history. Despite the lack of symptoms, histopathological analyses showed that all 66 individuals included in the published study (see item 4 below) showed evidence of Alzheimer's pathology. Specifically, 100% of the study subjects showed presence of tau pathology (a.k.a. neurofibrillary degeneration), as well as presence of dystrophic (senescent) microglial cells. This means that even though patients were non-symptomatic, both neuronal and microglial degeneration were ongoing. This finding is consistent with the known fact that sporadic Alzheimer's is marked by a prolonged period where the disease is not clinically apparent but microscopic lesions are present. While the extent of the microscopic lesions was quite variable amongst individuals, who ranged in age from 42 to 93, it was clear that the amount of brain pathology present increased with aging but never reached levels typically found in patients with clinically apparent dementia. Thus, it takes a substantial amount of brain pathology to cause symptomatic disease. Most interesting was the finding that only 23 out of 66 subjects included in the study showed presence of amyloid deposits, a finding that contradicts the prevailing hypothesis regarding Alzheimer's. The prevailing theory is called the amyloid cascade hypothesis, and states that extracellular deposits of amyloid are the cause of neurodegeneration. Obviously, if all 66 subjects show neurofibrillary degeneration, but only one-third shows amyloid, the amyloid theory cannot be true. Moreover, the amyloid theory also claims that amyloid causes neurodegeneration by triggering inflammation in the brain. In this cohort of 66 only five patients showed evidence of brain inflammation. This finding is also at odds with the amyloid hypothesis because it shows that inflammation cannot account for the neurodegeneration that is present in 61 of the 66 subjects studied. Thus, collectively the findings raise very significant concerns about the validity and relevance of the amyloid cascade hypothesis, which has dominated Alzheimer's research for the past 30 years. At the same time, these results are very consistent with the fact that every single therapeutic approach aimed at removing or suppressing amyloid formation has failed to show benefits in human clinical trials. This is important because it shows that Alzheimer's research must change direction and move away from the amyloid hypothesis because this theory is very likely to be false, and therapies targeting amyloid ought to be abandoned, as they are highly unlikely to help Alzheimer's patients. The work performed under this award supports an alternative hypothesis put forth by the PI, which is the microglial dysfunction theory. According to this theory, the gradual loss of microglial cell function (senescence) is what causes neurodegeneration because microglial support is essential to maintain a healthy neuronal population.

Follow On Funding: None at the time of reporting.

Collaborations: This collaboration is between the University of Florida and the University of Leipzig, Germany. Both institutions are involved in postsecondary education of graduate and medical students.

Currently, one graduate student and one medical student from the University of Leipzig are involved in this project.

Journals: Streit, W.J., Braak, Del Tredici, K., Leyh, J., Lier, J., Khoshbouei, H., Eisenlöffel, C., Müller, W. and Bechmann, I. Microglial activation occurs late during preclinical Alzheimer's disease. Glia 66:2550-2562, 2018.

Patents: None at the time of reporting.

17. **Grant #8AZ20:** Seeded Interactions of Abeta; And Neurofibrillary Tangle Pathologies in Mouse Models

    Principal Investigator: Guilian Xu, PhD

    Organization: University of Florida

    Progress Report: The purpose for this study is to develop methods to generate mice that more faithfully reproduce the pathology and symptoms of Alzheimer's disease (AD). Alzheimer's disease is characterized by abnormal accumulation of aggregated Abeta peptide and tau protein which form senile plaques and neurofibrillary tangles in the brain, respectively. The study builds on prior work in which research staff have made several different types of transgenic mice that express human genes associated with AD. Recently, research staff have demonstrated that the pathology that develops in the mouse models can be altered by injecting a small amount of brain tissue lysates from mice that develop Alzheimer amyloid pathology. These lysates contain amyloid plaques that can act as "seeds" to accelerate the onset of pathology and alter the appearance of pathology in the mouse models. Research staff have also determined that amyloid seeds can be generated from human brain tissues obtained from individuals that died of AD. These human seeds can be amplified by injecting into mice, providing a source for amyloid seeds that are derived more directly from humans. Interestingly, seeds from these different sources (existing AD mouse models or human AD brain) can produce distinct types of AD pathology when injected into transgenic mice that harbor genes associated with AD. Additionally, other work has shown that the neurofibrillary tangle pathology of AD can also be seeded in mice by injecting preparations of purified human tau protein (the main component of tangles). The purpose of this study was to test different combinations of seeds in three different mouse models to identify a combination that could robustly induce pathology in mice that more closely resembled human AD pathology. If successful, this study could develop animal models that are more predictive of human disease and provide a means to test new drugs more effectively. There remains an urgent need to develop better drugs for AD which is estimated to affect over 500,000 Floridians.

    Over the past year, research staff have been working with two different mouse models of Alzheimer amyloid pathology and one mouse model of Alzheimer neurofibrillary tangle pathology. These different mice have been bred to each other and then injected with the different types of seeds described above. In mice that have the potential to produce both amyloid and tangle pathology, the research staffs have demonstrated that injecting

amyloid seeds will accelerate the onset of amyloid pathology in the mice. More interestingly, preliminary data suggest that when amyloid pathology is accelerated, then tau pathology is also accelerated. This finding is potentially very impactful because a similar association between these pathologies is a hallmark of AD. Ongoing studies to produce and analyze additional mice will allow research staff to determine whether these preliminary findings can be confirmed.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

18. **Grant #8AZ22:** Cardiovascular and Lifestyle Stressors of Hippocampus and AD Related Brain Regions

    Principal Investigator: Noam Alperin, PhD

    Organization: University of Miami

    Progress Report: Late onset Alzheimer's disease (AD) is a multifaceted disorder with multiple lifestyle stressors contributing to its progression. It is now evident that the disease processes (e.g., deposition of toxic proteins in the brain) start years before onset of clinical symptoms. Research by others and by this group provided evidence that poor sleep quality is one of the main lifestyle stressors directly promoting progression to dementia. The group was the first to document that poor sleep quality is associated with accelerated tissue volume loss in AD-signature brain regions in elderly subjects, even before onset of cognitive symptoms. Elderly who are cognitively intact and suffer from poor sleep have significantly smaller AD-signature brain regions compared with a matching elderly cohort comprised of good sleepers. Not only did research find that substantial loss of brain tissue occurs early on, while subjects are still cognitively intact, staff further found that differences between the poor and good sleepers are larger in the cognitively intact cohort compared with a mild cognitive impairment cohort. This implies that the negative impact of poor sleep quality is more dominant in the earlier phase prior to onset of symptoms, and therefore intervention in that phase to improve sleep quality may have the most beneficial impact toward delaying onset of cognitive symptoms.

    Researchers further compared rates of tissue volume loss for the hippocampus (AD-critical region) over two years between poor and good sleepers. Preliminary results show that poor sleep quality accelerates the average rate of tissue loss in the hippocampus by a factor of two and a half compared to the rate in the good sleepers. Therefore, if this rate can be reduced by half, by improving sleep quality, theoretically, staff may delay onset of dementia by about two years. It is projected that in 25 years, one in every 45 Americans would have neurodegenerative dementia. Interventions that delay the disease onset by as little as two years could reduce the number of projected new cases

by 1.94 million. This not only will improve the quality of life for many elderly community dwellers, it would also have a significant economic impact.

A second aspect of this research is investigating the impact of a cardiovascular stressor. With aging, blood vessels become less elastic which results with a more pulsatile blood flow dynamic. This, in turn, causes the magnitude of the pulsating amount of blood volume in the brain during each cardiac cycle to increase. Therefore, as people age, the cerebral vasculature undergoes a higher level of stress which likely impairs the blood brain barrier (BBB), a mechanism protecting the brain from outside toxins. An impaired BBB is one of the factors leading to loss of brain health. This research tests for a possible link between the cerebral blood flow dynamics and rate of brain atrophy.

Follow On Funding: Florida Department of Health - $250,000 (pending).

Collaborations: None at the time of reporting.

Journals: Effect of sleep quality on amnestic mild cognitive impairment vulnerable brain regions in cognitively normal elderly individuals. **Alperin N**, Wiltshire J, Lee SH, Ramos AR, Hernandez-Cardenache R, Rundek T, Curiel Cid R, Loewenstein D. Sleep. 2019 Mar 1;42(3). pii: zsy254. doi: 10.1093/sleep/zsy254.

Patents: None at the time of reporting.

19. **Grant #8AZ23:** The Relationships Between Multimodal Neuroimaging Biomarkers and a Novel Cognitive Stress Test (CST) Among Ethnically Diverse Older Adults

    Principal Investigator: David Loewenstein, PhD

    Organization: University of Miami Miller School of Medicine

    Progress Report: This research study has assembled a unique consortium between top investigators from the University of Miami, University of Florida, Florida International University, and Mount Sinai Medical Center, generating an unprecedented opportunity to evaluate: a) a newly-developed computerized Cognitive Stress Test (CST) developed to identify unique cognitive markers of early Alzheimer's disease among diverse ethnic and cultural groups (African-American, Hispanic, and White-Non-Hispanic) at risk for Alzheimer's disease; and b) the use of study state-of-the-art multi-modal neuroimaging (tau PET/CT, amyloid PET/CT, Brain MRI to measure cortical thickness, regional brain volumes, and DTI).

    This consortium of experienced and productive investigators will be the first in the state of Florida to examine the relationship between the CST, tau and amyloid load in the brain as they relate to cognitive markers that have been found to be sensitive to detecting preclinical Alzheimer’s disease (AD) by uniquely tapping susceptibility to proactive semantic interference (PSI) and failure to recover from PSI (frPSI), and failure to recover from retroactive semantic interference (frRSI). The consortium will leverage existing resources and data provided by the 1Florida ADRC and the University of

Miami's longitudinal NIH study on aging and cognition (Dr. Loewenstein, PI) and will recruit additional diverse older adults at risk for early AD.

The collaborative team provides special expertise in quantitative multimodal neuroimaging, diagnosis of early cognitive impairment (MCI and PreMCI states), and the development of novel cognitive stress paradigms that are cross-culturally sensitive. This collaborative study is high impact in that it expands upon and further refines diagnostic strategies for early detection of preclinical AD and emerging treatments. It will also yield important and critical pilot data for successful collaborative R01 and other federal grant submissions to the National Institutes of Health.

There has been significant progress with this study. The computerized cognitive stress tests (CST) have been developed in English and Spanish and are being administered to persons with amnestic Mild Cognitive Impairment (aMCI), those who have PreMCI, and older adults who are cognitively normal representing diverse ethnic and cultural backgrounds. A contract with Life Molecular Imaging (formerly Piramel) has provided the study team with a tau reagent to image a subset of 30 participants. This study represents the first attempt in the country to relate these novel cognitive markers associated with amyloid to tau in the brain. During this reporting period, 19 study participants completed their CST test and obtained tau PET scans. The tau imaging infrastructure built is operating effectively and staff are making progress to meeting the study aims. Importantly, the work done as part of this grant contributed to a federal RO1 application that has received recent funding to continue to provide amyloid and tau imaging as it relates to performance on the CST among at-risk diverse individuals.

Follow On Funding: National Institute on Aging - $2.6 million.

Collaborations:

University of Florida: Stephen Dekosky MD - Tau and Amyloid Neuroimaging Expert

Mount Sinai Medical Center Alzheimer's Disease: Maria Grieg MD - Cross-Cultural Diagnosis of Early AD

Florida International University: Makek Adjouadi, PhD - Cate Center for Neuroimaging

Postdoctoral Researchers: Three postdoctoral research fellows are currently collaborating in the research activities.

Nova Southeastern University, College of Psychology: Three doctoral students from the Clinical Psychology Program are assisting in the research activities as part of their practicum experience program.

Carlos Albizu University. One doctoral student from the Clinical Psychology Program is assisting in the research activities as part of their practicum experience program.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

20. **Grant #8AZ24:** Extracellular Vesicles as Novel Therapeutic Targets in Alzheimer's Disease

    Principal Investigator: Michal Toborek, MD, PhD

    Organization: University of Miami

    Progress Report: Virtually all cells of the human body shed vesicles into the extracellular space, which then travel via the blood stream and can reach distant organs. These vesicles, named “extracellular vesicles” (ECVs), carry content characteristic to the cells they originate from, including a protein called amyloid beta (Abeta). Deposits of Abeta in the brain have been linked to the memory loss and cognitive decline in individuals suffering from Alzheimer’s disease (AD).

    The mechanistic link between elevated deposits of Abeta in the brain and loss of memory in AD is not fully understood. This project is based on the hypothesis that ECVs carrying Abeta can deliver this cargo to neural progenitor cells (NPC), i.e., cells that produce new neurons in the adult brain. NPC-derived neurons are critically important for normal brain function because they are built into normal neuronal networks and participate in memory formation.

    This project explores not only the role of ECV in Abeta transfer to NPC but also the outcomes of this process, such as impaired production of new neurons and induction of inflammatory responses. By better understanding of these events, staff will be able to provide novel therapeutic targets in AD. Thus, the proposal is highly significant to Floridians suffering from AD and/or their families. If successfully completed, the research will constitute an excellent return on investment.

    Work on the project progresses as planned. During the reporting period, staff focused on the role of receptor for advanced glycation end products (RAGE) in ECV-mediated transfer of Abeta to NPCs. Blocking RAGE with a high-affinity specific inhibitor (FPS-ZM1) resulted in diminished Abeta uptake by NPCs. Interestingly, the novel data demonstrated that ECV-mediated Abeta transfer to NPCs was associated with induction of inflammasome proteins, such as NLRP3, a major pattern recognition receptor that is expressed in response to a variety of stimuli, and the adaptor protein called apoptosis-associated speck-like protein containing CARD (ASC). NLRP3-positive immunoreactivity was mostly nuclear with a finer granular pattern. In addition, a small number of brighter cytoplasmic or extracellular immunoreactivity dots were apparent in all experimental groups. Regarding ASC expression, its immunoreactivity was distributed overwhelmingly in the nuclear area with an intense granular pattern consisting of different sized nuclear granules in control cells. Following ECV exposure, this pattern remained primarily nuclear; however, several bright punctate immunoreactivity spots localized to the cytoplasm or the extracellular space appeared. These exciting results indicated also that nuclear colocalization of Aβ with inflammasome proteins was regulated by RAGE. In the last series of experiments, staff evaluated the impact of ECVs carrying Abeta on

differentiation of NPCs to mature neurons. The most important results provided the evidence that the number of mature neurons was markedly diminished by exposure to ECVs with Abeta. Overall, the obtained results fully support the leading hypothesis of the funded grant and provide a foundation for further studies. The results are currently being prepared for publication and a manuscript will be submitted soon.

Follow On Funding: None at the time of reporting.

Collaborations: This proposal resulted in collaboration with Dr. Marta Garcia Contreras from the Diabetes Research Institute at the University of Miami School of Medicine on analysis of extracellular vesicles, and with Dr. Shanta Dhar from the Department of Biochemistry and Molecular Biology, University of Miami School of Medicine.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

21. **Grant #8AZ25:** Identification of Noncoding Functional Variant(s) Underlying Alzheimer Disease GWAS Hits

    Principal Investigator: Anthony Griswold, PhD

    Organization: University of Miami

    Progress Report: Alzheimer's disease (AD) is the most common neurodegenerative disease and the leading cause of dementia with ~510,000 individuals in Florida (nearly 12% of Florida's senior population) and approximately 5.4 million individuals in the US across all racial and socio-economic strata. Current and emerging AD therapies focus on treating clinical symptoms, not the underlying pathological mechanisms of disease, largely because the field's understanding of these mechanisms is still limited. In an era of personalized medicine where one's disorder will be treated based not just on specific clinical symptoms but underlying genetic information, it is imperative the genetics field makes a concerted effort to understand the biological mechanisms in which genetic (risk) factors exert their effect.

    Genome wide association studies (GWAS) have identified at least 20 genetic markers associated with AD. The vast majority of associated GWAS variants (~77%) in complex diseases are located in non-protein coding regions of the genome with potential regulatory function. Identification of the functional variants contributing to risk in noncoding regions is complex. First, since SNPs in association studies are part of large linkage disequilibrium (LD) blocks containing multiple SNPs, the top associated SNP may not be the 'driver' variant, rather any of the variants in LD with the top SNP could be the variant driving the association. Second, the regulatory elements (RE) including promoters, enhancers/silencers and insulators can affect genes located a significant distance away depending on 3D conformation of the chromosome. Taken together, the gene nearest to the top SNP may not be the gene whose affected expression is increasing risk for AD, complicating interpretation of GWAS results.

This proposal sought to identify the functional variant(s) driving the association in the PICALM locus. Studies in different racial and ethnic population groups have found evidence for association with AD in white/non-Hispanic, African American, and Hispanic datasets (Naj et al., 2011; Lee et al., 2011; Logue et al., 2011). However, sequencing of the coding region of PICALM has not identified coding variants of great effect. Toward understanding the role of variants in the PICALM locus in regulating downstream mechanisms, the research team utilized extensive in-silico bioinformatics analysis to annotate more than 20,000 variants identified across diverse populations in genome sequencing studies. Staff have begun to analyze data generated as part of a massive parallel reporter assay (MPRA) to identify candidate variants that regulate gene expression. The future of this project will use genome-editing technology combined with RNAseq and AD phenotype analysis to determine the effects of these variants. The development of an in-house MPRA protocol will be widely applicable, not only to the PICALM locus, but to other noncoding regions reported to be contributing to AD and by extension other neurodegenerative disorders.

Follow On Funding: None at the time of reporting.

Collaborations: During the planning of the reporter assay in Aim1 of this study, researchers contacted RYAN TEWHEY, Ph.D. currently at Jackson Laboratory and previously at Harvard University. Dr. Tewhey was one of the original people to publish using this technique (PMID: 27259153) and his role was purely advisory, assisting in the development of parameters for probe design and molecular techniques. He will be acknowledged on future manuscripts from the data derived from this project.

Similarly, staff have discussed the project with Casey Brown, Ph.D. at the University of Pennsylvania. The group is involved in several similar studies related to other regions and diseases, so staff have exchanged thoughts related to the bioinformatic and laboratory procedures related to MPRA analysis. Dr. Brown is also a main contributor to the GTEx consortium on regulatory variants and has been a welcome collaborator.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

22. **Grant #8AZ26:** Investigating the Role of SORL1 in Alzheimer's Disease

Principal Investigator: Derek Dykxhoorn, PhD

Organization: University of Miami

Progress Report: Alzheimer’s disease (AD) is the leading cause of dementia among the elderly affecting ~500,000 Floridians. This disease places a significant social and economic burden on families and the healthcare system. Genetics studies over the past decades have identified a variety of AD-associated genes. How many of these genes contribute to the development of AD is a point of ongoing research. This proposal seeks to understand the role that variation in the Sortilin Related Receptor 1 (*SORL1)* gene

plays in AD pathogenesis. The SORL1 gene is able to function as a transmembrane signaling receptor and can bind low density lipoproteins as well as play a role in endocytosis and intracellular sorting. Given the importance of intracellular trafficking in the proper process of amyloid precursor protein (APP), a key protein in the pathology of AD, researchers hypothesize that variants in SORL1 will affect the production of pathogenic cleavage products of APP.

Researchers had previously identified a novel single nucleotide deletion causing a premature stop codon in a family with early onset AD. Induced pluripotent stem cells (iPSC) have been generated from whole blood collected from members of this family. These iPSC were shown to be heterozygous for the single nucleotide deletion. To understand the role of this deletion on AD pathogenesis, staff propose to correct the mutation in the iPSC lines which bear the mutation and add the mutation to lines which lack the mutation using genome editing technologies. This will allow for the determination of both the role of SORL1 in AD pathology and establish whether variants in the SORL1 gene are sufficient to induce AD-associated phenotypes. To that end, staff have derived the vectors for the genome editing and tested these vectors in iPSC lines for their efficiency of cutting and potential for effective genome editing. Staff are working on improving these metrics by optimizing the ease with which these constructs can be targeted to the cells and have taken the lines from the patient samples and age and gender matched controls and differentiated these cells into different populations of brain cells, including cortical neurons, astrocytes, and microglial cells. Staff are in the process of testing these different cell types to identify the impact that this mutation has on the production of the pathogenic protein amyloid beta, processing of these protein products, the production of phosphorylated tau protein (another pathogenic molecule found in Alzheimer disease), and the activation of the immune cell through engagement of microglia cells. These iPSC lines will be differentiated into cortical neurons for analysis of AD-associated cellular phenotypes, Understanding the role that the SORL1 gene plays in AD pathogenesis will elucidate the molecular mechanisms that underlie AD and help in developing more effective strategies to treat this common and debilitating disease.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

23. **Grant #8AZ27:** Emerging Role of Tau Citrullination in Alzheimer's Disease

    Principal Investigator: Maj-Linda B. Selenica, PhD

    Organization: University of South Florida

Progress Report: The laboratory moved to University of Kentucky on August 19th, 2019. All frozen tissue was stored at -80C freezer that was purchased by this grant and is located in the College of Pharmacy/USF. The PI will arrange for transfer of tissue once a new -80C is purchased.

AIM 1 DESCRIPTION. To determine if repression of PAD4 reduces tau citrullination in an animal model of tauopathy. Researchers hypothesize that repression of PAD4 during tauopathy decreases tau citrullination and mitigates the tau phenotype. The AAV viruses are injected into rTg4510 tau transgenic mice and non-transgenic littermates (n=10/group) to downregulate neuronal PAD4 expression and determine tau citrullination levels.

All brain is dissected in seven brain regions and stored at -80C for Western blot assay. Tissue has been homogenized and will be used to measure the levels of the citrullination and phosphorylation phenotype will be performed following previous established protocols in the laboratory.

AIM 2 DESCRIPTION. To determine if immunization against tau-citR230 subverts tau neuropathology in an animal model of tauopathy. Researchers postulate that citrullination of tau at R230 exposes neo epitopes, which enhance immunogenicity and promote tau pathology. Staff have immunized rTg4510 tau transgenic mice against tau-citR230.

Research staff measured by ELISA the antibody levels in the serum of mice injected as described. Staff found out that immunization with both citR230 and pT231, that both ntg and rTg4510 produced antibody titers at the range of 36571vs. 482857 for citR230 tau. Similarly, a difference in the antibody production was found between both genotypes when injected with pT231, 65714 vs. 13571, respectively.

Brain fractionation of all animals was also performed during that period. Staff have collected protein fraction from S1, soluble tau, S2, sarkosyl soluble tau and P3, sarkosyl insoluble tau, to measure levels of tau-citR230 as primary outcome.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

24. **Grant #8AZ28:** Microglial Phenotype in Alzheimer's Disease.

Principal Investigator: Kevin Nash, PhD

Organization: University of South Florida

Progress Report: The purpose of this grant is to study and characterize the microglial cells (immune cells of the brain) that are present in the Alzheimer’s model termed

Tg4510. This model has increased tau protein which cause neuron loss as well as increased inflammation in the brain. This study is focused on the inflammation. The study has completed the isolation of the microglia and extracted RNA from the test animals. The RNA was sent out for complete analysis and the data recently received. A comparison of the changes occurring to the microglial cells during disease compared to the normal mice is currently under way. A comparison will also be done with the additional treatment group which received the anti-inflammatory gene therapy. This will be the first extensive comparison of both protein producing gene expression as well as non-protein producing RNA, termed long-noncoding RNA. Staff believe that this information will be critical in understanding how inflammation is changing in Alzheimer's and how an anti-inflammatory approach may correct this.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

25. **Grant #8AZ29:** Divergent RanBP9 Signaling in Tau Pathogenesis

Principal Investigator: David E. Kang, PhD & Jung A. Woo

Organization: University of South Florida

Progress Report: Alzheimer's disease (AD) is a devastating neurodegenerative dementia associated with Abeta and tau pathologies in brain that currently afflicts 5.4 million individuals in the United States and close to 500,000 in the state of Florida. Due to the aging population, the number of cases for the United States overall is estimated to rise to 13.2 million by 2050 if no effective treatment is found. In Florida, it is estimated that the number of individuals living with AD is rising by 20% every 10 years, thereby reaching a number close to 1.2 million by the year 2050. In 2012, the cost of caring for AD and related dementia patients in the United States stood at $200 billion/yr, and this figure is projected to rise to $1.1 trillion/yr by 2050 (2012 dollars). Therefore, AD is a tremendous cost to patients and families in both human and financial terms. The cost of doing nothing is far greater than the cost of doing research to prevent or slow the progression of AD. The major hypothesis of AD is the Abeta/amyloid hypothesis, which states that the accumulation of Abeta/amyloid is an early and necessary event in the pathogenic progression that promotes tau pathology and associated neuronal degeneration. Indeed, this hypothesis is widely supported by a multitude of genetic, biochemical, and cell biological studies. However, it is also clear that tau is required for Abeta/amyloid to transmit its neurotoxic signals, and accumulation of tau *per se* is neurotoxic. Therefore, understanding the molecular mechanisms that regulate the production of Abeta and how neurotoxic signals are transmitted to tau are critical for discovering novel and promising therapeutic strategies to combat AD. Thus far, no disease-modifying drug has yet to show efficacy in moderate to severe cases of AD,

perhaps because a therapeutic strategy may need to treat both ongoing Abeta production and block the neurotoxic signals between existing Abeta and tau pathologies.

The molecular pathway under investigation (RanBP9 & cofilin) regulates both new Abeta production and Abeta/amyloid-induced tau pathogenesis. Research findings indicate that RanBP9 not only activates cofilin to deregulate tau/microtubule dynamics but also directly promotes tau stability by positively regulating chaperones Hsp90 and Hsc70. This proposal seeks to understand how RanBP9 together with Hsp90/Hsc70 regulate tau aggregation and microtubule dynamics as well as investigate whether activated cofilin (but not inactive cofilin) promotes tau pathology in brain. Both aims have profound implications for AD therapeutics, as Hsp90/Hsc70 and cofilin activation are molecular therapeutic targets for AD under active development. The findings thus far indicate that the RanBP9 molecule positively regulates tau levels, which is one hallmark of AD pathology. Staff are currently testing whether RanBP9 also promotes tau aggregation or clumping together. Finally, staff are also testing whether the 'activated' form of cofilin versus the 'inactive' form selectively promotes tau pathology in brain. This question has important therapeutic implications, as controlling the 'activation' of cofilin can be done using chemical inhibitors to an enzyme called SSH1, which is currently being developed.

Follow On Funding: None at the time of reporting.

Collaborations: USF Health: Yan Yan, graduate student

Journals: Woo JA*, Liu T, Fang CC, Cazzaro S, Kee T, LePochat P, Yrigoin K, Penn C, Zhao X, Wang X, Liggett SB, Kang DE* (2019). Activated cofilin exacerbates tau pathology by impairing tau-mediated microtubule dynamics. Commun Biol. 2019 Mar 22; 2:112. doi: 10.1038/s42003-019-0359-9. eCollection 2019.

Liu, T., Woo, J.A., LePochat, P. Yan, Y., Kang, D.E (2019). Dual Role of Cofilin in APP trafficking and Amyloid β Clearance. FASEB J. PMID: 31646885 DOI: 10.1096/fj.201901268R.

Kang, D.E., Woo, J.A. (2019). Cofilin, a master node regulating cytoskeletal pathogenesis of Alzheimer's disease. J Alzheimers Dis, PMID:31594228 DOI:10.3233/JAD-190585.

Patents: None at the time of reporting.

26. **Grant #8AZ30:** Exploiting GPRC6a Antagonists to Mitigate Tau Deposition

Principal Investigator: Daniel C. Lee, PhD

Organization: University of South Florida

Progress Report: Over the reporting period of up to September 30, 2019 researchers have made progress on several fronts. Staff have procured tissue samples from the brains of Alzheimer's disease (AD) or aged matched controls and requested brain tissue from the NIH brain bank, which consist of different repositories around the United States.

To determine if GRPC6a expression is altered in the brains of Alzheimer's disease staff measured GRPC6a by western blot analysis from whole cell homogenates. The samples included (ages approximately 65-100) 10 aged matched controls and 10 samples from clinically diagnosed Alzheimer's disease brain. Staff also gather information on Braak Stages, which ranged from (I-III for controls) and III-VI for AD samples. Staff tested different antibodies that recognize different putative GPRC6a isoforms (isoform 1 and isoform 3). Overall, both isoforms for GPRC6a were significantly increased in the AD samples, suggesting that AD pathology promotes dysregulation of GPRC6a. Animal studies regarding the gene repression of GPRC6a in tau transgenic mice (shRNA-GPRC6a vs control-GFP) are continuing to be collected and analyzed. Researchers have initial evidence that reducing GPRC6a expression improves some cognitive measures in tau transgenic mice. Regarding additional mechanisms and functionality of GPRC6a staff performed experiments in which the GPRC6a receptor was overexpressed in Hek293 cells. To understand if simple increase in receptor expression promotes mTORC1 activation and tau, staff used inducible tau in HEK293 cells, which is argued to not express GPRC6a on the cell surface. Staff overexpressed GPRC6 at different plasmid concentration along with GFP as a control plasmid and observed a general increase in tau levels, phospho tau s214, mTOC1 substrates, and ULK757 following the GPRC6a overexpression. These data suggest that simply increasing expression levels of GPRC6a can promote mTORC1 signaling. To compliment these findings, staff observed increased GPRC6A expression in rTg4510 mice indicating that the tau phenotype promotes aberrant GPRC6a expression. Lastly, to understand if the novel GPRC6a allosteric antagonist impacts tau biology through oligomerization staff created a new cell line from mouse N2A cells that stably expresses two tau constructs fused to split GFP. Only upon tau dimerization or oligomerization does GFP recombine for complementation to fluoresce. This cell line allows researchers to measure tau oligomerization and treatments or pathways that impact tau biology that facilitates or decreases tau oligomerization. Research staff tested whether different concentrations of Drug 47661 for 72hrs (the lead GPRC6a allosteric antagonist) impacts tau oligomerization in the novel N2AssGT cell line and found that concentrations (3-30uM) reduce GFP fluorescence. This suggest that GPRC6a antagonists reduces clearance of tau oligomers or at least clears tau to a degree that alters/ reduces tau oligomer formation. Overall, the data for the past year shows that AD brains and tau brains show increased levels of GPRC6a receptor expression. Initial data indicates that reducing GPRC6a receptor expression in the brains of tau transgenic mice using gene therapy improves some cognitive impairment. Finally, the novel GPRC6a antagonist not only clears monomeric tau but also reduces oligomeric tau, suggesting that GPRC6a could be a potential treatment for AD and tauopathies.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: USF Ref. No.: 16B142 - Exploiting Allosteric Antagonists to GPRC6a to Mitigate Proteinopathies *Patent pending.*

27. **Grant #8AZ32:** Longitudinal Assessment of BDNF Levels with Bacopa Monnieri Treatment in those at Risk of Developing Alzheimer's Dementia

    Principal Investigator: Andrew Keegan, MD

    Organization: The Roskamp Institute

    Progress Report: This two-year project began in March of last year (2018) with the first component. The goal of that first portion was to evaluate how a population of subjects who are at risk for developing Alzheimer's Disease (based on age) have changes in levels of a factor important for maintaining neurons and their connections called Brain Derived Neurotrophic Factor (BDNF). As of the end of April 2019, research staff completed collection of blood samples and memory tests for those subjects (86 in total) and are compiling the data analysis and preparing a manuscript.

    For the second component of the study, staff began a clinical trial, after obtaining IRB (Institutional Review Board) approval whereby subjects were supplied a supplement (Bacopa) over a 3-month period and changes in Brain Derived Neurotrophic Factor (BDNF) will be assessed. Staff have recruited the first approximately 25% of proposed subjects and are working to complete those recruitment goals over the next several months. These will be local Floridians participating in the study that may provide preliminary data on the potential benefits of this supplement on cognitive aging.

    Follow On Funding: None at the time of reporting.

    Collaborations: There is one consultant, Prof. Con Stough, Ph.D. from Swinburne Centre for Human Psychopharmacology, Swinburne University, Australia.

    Journals: None at the time of reporting.

    Patents: None at the time of reporting.

APPENDIX D
FISCAL YEAR 2019-2020 ACTIVE GRANTS
(Funding Year 2016-2017)

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
7AZ11	University of Central Florida	Sugaya, Kiminobu	$ 100,000	$ 64,702	$ 35,298	2/06/2017	3/31/2021	No	Yes	No
7AZ21	Mayo Clinic Jacksonville	Cook, Casey	$ 250,000	$ 192,310	$ 57,690	2/01/2017	3/31/2020	No	Yes	No
7AZ22	Mayo Clinic Jacksonville	Kanekiyo, Takahisa	$ 250,000	$ 147,060	$ 102,940	2/01/2017	3/31/2021	No	No	No
7AZ26	University of Miami	Wahlestedt, Claes	$ 100,000	$ 64,702	$ 35,298	2/01/2017	3/31/2021	No	Yes	No
7AZ27	University of Central Florida	Tatulian, Suren	$ 100,000	$ 84,612	$ 15,388	2/01/2017	3/31/2020	No	Yes	Yes

Active Grants Fiscal Year 2018-2019
(Funding Year 2016-2017)

1. **Grant #7AZ11:** Antibody Targeting of IL1RAP and Studying their Therapeutic Effects in Mouse Models of Alzheimer's Disease

   Principal Investigator: Kiminobu Sugaya, PhD

   Organization: University of Central Florida

   Progress Report: This grant proposal is to treat Alzheimer's disease using exosomal delivery of antibody against the interleukin-1 receptor accessory protein (IL1RAP), which has been associated with an increase in amyloid plaque accumulation. Exosomes are small vesicles secreted by cells containing cellular proteins, nucleic acids, and lipids. They consider to be playing a role in intercellular communication. Since they are 30 to a few hundred nm size and made of lipid membrane, they can pass through blood brain barrier (BBB), which prevent us from delivering many drugs to the brain.

   Accomplishments during the reporting period:

   a. Established optimal isolation and purification of exosomes from human mesenchymal stem cells by magnetic beads conjugated with antibody against CD63, which is the exosomal surface marker.
   b. Established method to attachment of signal peptides to the surface of exosome using click chemistry.
   c. Recognized an increased efficacy of exosomal delivery to the brain by attaching brain homing peptide to the exosomes' surface, which is recognized by BBB and selectively captured into the brain from the blood, to the surface of exosomes. This technology allows staff to deliver of the cargo to the brain by intravenous injection of the modified exosomes.
   d. Recognized an increased efficacy of exosomal delivery to the neural cells by attaching neural cell adhesion molecule (NCAM) to the exosomes' surface, which is recognized by neural cell surface protein and selectively captured into the neural cells. This technology allows the delivery of the cargo to the neural cells by intravenous injection of the modified exosomes.
   e. Inserted a variety of proteins to the exosomes as a cargo using special type of protein expression vector. This technology allows input of antibody and/or part of antibody to the exosome.
   f. Established induced pluripotent stem (iPS) cell culture from control and Alzheimer's disease subjects.
   g. Optimized the differentiation condition for the iPS cells to neural stem cells (NSCs) forming neuron-sphere.
   h. Optimized the differentiation condition for NSCs to differentiate into the neural cells, such as neurons, oligodendrocytes, and astrocytes.

i. Established three dimensions (3D) culture system using hydrogel scaffold, which mimics human brain tissue except microglia. Staff are currently developing the differentiation process from iPS cells to the microglia.

This project has experienced the ability to produce in vitro 3D models of AD and control brain in vitro and currently staff are establishing BBB in vitro model the by combination of human endothelial cells and astrocytes. If these two different models are combined, human BBB and AD and control 3D in vitro model, there will be the ability to build in vitro model human brain with BBB to test protection efficacy of the modified exosomes.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Vida, M., Bacchus, M., and Sugaya, K., Differential sequences of exosomal NANOG DNA as a potential diagnostic cancer marker. PLOS ONE, 2018, 13(5): e0197782. https://doi.org/10.1371/journal.pone.0197782

Vida, M., Bacchus, M., and Sugaya, K., Differential Sequences and Single Nucleotide Polymorphism of Exosomal SOX2 DNA in GBMPLOS ONE 2019 submitted.

Patents: None at the time of reporting.

2. **Grant #7AZ21:** Evaluating the Mechanism By Which Tau A152t Modulates Risk of Tauopathy

Principal Investigator: Casey Cook, PhD

Organization: Mayo Clinic Jacksonville

Progress Report: Pathogenic mutations in the tau gene are linked to the onset of tauopathy, but the A152T variant is unique in acting as a risk factor for a range of disorders including Alzheimer's disease (AD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and dementia with Lewy bodies (DLB). To provide insight into the mechanism by which A152T modulates disease risk, a novel mouse model was developed and validated by confirming the distinct biochemical features of A152T tau in postmortem brain tissue from human carriers. Specifically, Tau^{A152T}-AAV mice exhibited increased tau phosphorylation that remained localized to the soluble fraction. To investigate the possibility that the A152T variant might alter the phosphorylation state of tau on T152 or the neighboring T153 residue, a novel antibody was generated that revealed significant accumulation of soluble tau species that were hyperphosphorylated on T153 (pT153) in Tau^{A152T}-AAV mice. Providing new insight into the role of A152T in modifying risk of tauopathy, as well as validating the Tau^{A152T}-AAV model, the results demonstrate that the presence of soluble pT153-positive tau species in human postmortem brain tissue differentiates A152T carriers from noncarriers,

independent of disease classification. These findings implicate both pT153 and an altered solubility profile in the mechanism by which A152T modulates disease risk and, were accepted for publication in *Acta Neuropathologica Communications*.

To determine whether pT153 is required for A152T-associated toxicity, mice were then injected with either Tau^{A152T}-AAV, $Tau^{A152T/T153A}$-AAV (to prevent phosphorylation at T153), or Tau^{T153E}-AAV (to mimic pT153). Behavioral analysis revealed abnormalities in both Tau^{A152T}-AAV and Tau^{T153E}-AAV mice, consistent with the idea that increased pT153 contributes to toxicity associated with A152T expression. There was also a significant reduction in brain weight in mice injected with Tau^{A152T}-AAV and Tau^{T153E}-AAV relative to GFP-AAV control mice, while there was no significant difference in brain weight between GFP-AAV and $Tau^{A152T/T153A}$-AAV-injected animals, in agreement with a protective role of T153A in reducing A152T toxicity. These results support the hypothesis that pT153 is required for A152T-induced toxicity, and also demonstrate that mimicking pT153 is sufficient to drive toxicity even in the absence of the A152T variant. Moreover, total human tau protein and mRNA levels were comparable across groups, verifying that the consequences of manipulating the A152 and T153 residues were not due to differences in expression levels.

Finally, to assess whether the mechanism by which the A152T variant increases tau phosphorylation at other epitopes is dependent on pT153, the amount of CP13 (phosphorylated S202)-positive tau was evaluated. As the presence of the T153A mutation in the A152T/T153A construct prevents pT153, the observation that tau phosphorylation at the CP13 epitope is significantly reduced in $Tau^{A152T/T153A}$-AAV relative to A152T-AAV mice is consistent with the idea that A152T acts to prime tau for phosphorylation in a mechanism that involves pT153. In contrast, Tau^{A152T}-AAV and Tau^{T153E}-AAV-injected mice exhibit similar levels of CP13-positive tau, indicating that mimicking pT153 recapitulates the biochemical phenotype observed in A152T-expressing mice, supporting the idea that pT153 is critical to the mechanism by which A152T exerts toxicity.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Carlomagno Y, Chung DC, Yue M, Kurti A, Avendano NM, Castanedes-Casey M, Hinkle KM, Jansen-West K, Daughrity LM, Tong J, Phillips V, Rademakers R, DeTure M, Fryer JD, Dickson DW, Petrucelli L, Cook C. Enhanced phosphorylation of T153 in soluble tau is a defining biochemical feature of the A152T tau risk variant. Acta Neuropathol Commun 2019 Jan 23;7(1):10. PMID: 30674342. https://rdcu.be/bh6Vy

Patents: None at the time of reporting.

3. **Grant #7AZ22:** ApoE and Cerebrovascular Aging in Alzheimer's Disease

Principal Investigator: Takahisa Kanekiyo, MD, PhD

Organization: Mayo Clinic Jacksonville

Progress Report: As the cerebrovascular system plays a critical role in maintaining brain homeostasis, disturbances of this pathway can lead to cognitive impairment and dementia during aging. Interestingly, population-based epidemiologic studies have revealed that cerebrovascular damage due to hypertension, diabetes or smoking is also associated with the increased risk for Alzheimer's disease (AD). While AD and vascular cognitive impairment and dementia (VCID) are major causes of dementia, the prevalence and the incidence of both diseases remarkably increase in an age-dependent manner. Although the process of aging is complex, current evidence indicates that aging is caused by the accumulation of senescent cells, where the increase of $p16^{INK4a}$ is the major hallmark. Thus, researchers investigated how the forced expression of $p16^{INK4a}$ in vascular endothelial cells impacts cerebrovascular function, using an in vivo gene delivery system through a unique recombinant adeno-associated virus 2 (rAAV2) with a modified capsid. Staff confirmed that intraperitoneal injection of the rAAV2, harboring an enhanced green fluorescent protein (EGFP) reporter gene under the control of the CAG promoter, successfully induced the specific expression of EGFP in cerebrovascular endothelial cells in mice. When $p16^{INK4a}$ or EGFP was expressed in cerebrovascular endothelial cells in wild-type mice at three months of age, results showed an increase in IgG leakage into the brain parenchyma in the $p16^{INK4a}$-expressed mice in comparison with the control EGFP-expressed mice, one month after the rAAV2 injection. Whereas staff also examined the coverage of endothelial cells with pericytes and major tight junction proteins six months after injection, there were no evident differences between two mouse groups. When cerebral blood flow was measured by in vivo 2-photon imaging 2.5 months after the rAAV2 injection in the mice, staff found that cerebrovascular endothelial $p16^{INK4a}$ overexpression substantially reduced blood flow in arterioles and capillaries in the cortex. Besides, staff confirmed the impaired spatial memory and learning six months after the rAAV injection. Together, the results indicate that p16INK4a-induced endothelial senescence disturbs cerebral blood supply as well as blood-brain barrier (BBB) integrity which may contribute to the pathogenesis of AD and VCID. Since APOE genotypes have been shown to influence cerebrovascular functions as well as cognitive performances, staff will continue efforts to determine how cerebrovascular senescence and APOE trigger the pathogenic cascade for age-related cognitive decline using mouse models and the unique AAV approaches.

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 #7AZ26:** Preclinical Investigation of an Optimized Formulation of Resveratrol, JOTROL, for Alzheimer's Disease

   Principal Investigator: Claes Wahlestedt, MD, PhD

   Organization: University of Miami Miller School of Medicine

   Progress Report: The purpose of this project is to investigate whether a novel formulation of the natural product resveratrol, named JOTROL, will be efficacious at mitigating Alzheimer's disease-like pathogenesis. Since the last legislative report, additional experiments were conducted and data were obtained from aged transgenic Alzheimer's disease mice that were purchased with funds from this grant to proceed with the aims. Here, effects of daily oral JOTROL treatment were investigated on the expression of the pro-inflammatory cytokines Interleukin-6 (IL-6), associated with depression and neuro- inflammation – and tumor necrosis factor alpha (TNF-alpha) – a well-documented player in the cytokine cascade during inflammatory responses and shown to be elevated in the blood of Alzheimer's disease patients. Here, 50 mg/kg of JOTROL is shown to significantly decrease both IL-6 and TNF-aplha expression. The effects of daily oral JOTROL treatment were also investigated on splenomegaly – an enlarged spleen condition observed in Alzheimer's mice, indicative of increased inflammation status – observed in Alzheimer's patients. Since significant decreases in the pro-inflammatory cytokines tumor necrosis factor alpha (TNF-alpha), and interleukin-6 (IL-6) were observed, The formulated hypothesis was that JOTROL would reduce overall inflammation associated with Alzheimer's disease and help mitigate disease progression. Indeed, researchers observed a decrease of spleen size in Alzheimer's mice treated with 50 mg/kg of JOTROL, compared to vehicle controls. As mentioned in the previous report, a challenge has been the low budget for this project, because small batches of mice have to be ordered at a time, and it has not been possible to purchase old/aged mice. Mice are thus bought young and allowed to age in these facilities. This does impact the pace of the project, but nonetheless, interesting data are being obtained. Animals are still being treated and data collected. The aims of the grant are expected to be completed in a timely manner. In the last legislative report, the possibility of using JOTROL to reverse Alzheimer's disease phenotype was covered. Currently, animals are being treated to investigate whether JOTROL can be used to prevent AD-like phenotype.

   Follow On Funding: None at the time of reporting.

   Collaborations: This reporting period, one undergraduate student from the University of Miami Coral Gables Campus (Ian Newman) and three graduate students (pre-doctorate) from the Departments of Pharmacology (Karolina Janczura, recently graduated), Biochemistry (Natalie Ricciardi) and Neuroscience (Jessica Dennison) at the University of Miami Miller School of Medicine performed research on this project. Staff are also constantly collaborating with the Florida-based startup, Jupiter Orphan Therapeutic, the providers of JOTROL.

Journals: Janczura K.J., Volmar CH, Wahlestedt C., Purification of H3 and H4 Histone Proteins and the Quantification of Acetylated Histone Marks in Cells and Brain Tissue, J Vis Exp., 2018, Nov 30;(141). doi: 10.3791/58648 PMID: 30582583.

Patents: None at the time of reporting.

5. **Grant #7AZ27:** Structure and Toxicity of Amyloid Beta Hetero-Oligomers

   Principal Investigator: Suren A. Tatulian, PhD

   Organization: University of Central Florida

   Progress Report: During the reporting period, progress has been made towards Specific Aims 1 and 2.

   Specific Aim 1: Circular dichroism (CD) and Fourier transform infrared (FTIR) studies have been conducted to identify the structures of Ab_{1-40} and its pyro glutamylated counterpart, $AbpE_{3-40}$. CD spectra of the peptides in hexafluoro isopropanol (HFIP) indicate a combination of unordered and a-helical conformations. Removal of the solvent caused transition to a-helical structure. To monitor the dynamics of structural changes upon hydration and to capture the intermediate structures, D_2O vapor was used to slow down the hydration. FTIR spectra of Ab_{1-40} and $AbpE_{3-40}$ in dry state indicating a-helical structure. Exposure of the peptides to D_2O vapor resulted in reduction of the a-helical signal and enhancement of the b-sheet component, signifying a-helicx to b-sheet structural transition.

   Fluorescence experiments have been conducted to detect aggregation of Ab peptide based on fluorescence resonance energy transfer (FRET) between the intrinsic fluorophores of the peptide. Since Ab is devoid of tryptophan, Phe-to-Tyr FRET was used. FRET has been detected for fragments carrying one or the other fluorophore, as evidenced by decrease in Phe emission (~280 nm) and increase in Tyr emission (~310 nm). Increase in thioflavin-T fluorescence occurred at later stages of incubation of the peptides in buffer.

   To identify the segments of Ab that initiate the aggregation and fibrillogenesis of the peptide, the aggregation and accompanying structural changes of overlapping peptide fragments have been studied. FTIR spectra of peptides Ab_{1-10}, Ab_{6-15}, Ab_{16-25}, and Ab_{26-36} identified a mixture of unordered and b-turn structures. The peptide Ab_{11-20} displayed b-sheet/b-turn structure, and peptides Ab_{21-30} and Ab_{31-42} demonstrated broad, featureless amide I bands of increased intensity. These data suggest that the 11-20 segment of the Ab peptide initiates the aggregation and fibril formation.

   Specific Aim 2: Solid state NMR (ssNMR) studies have been conducted to determine the structures of Ab_{1-42} and $AbpE_{3-42}$ in lipid environment. Since the cytotoxicity of Ab involves membrane binding and permeabilization, these studies would help understand the mode of membrane perturbation and pore formation by Ab peptide. The 1D ^{13}C

ssNMR spectra of both peptides, ^{13}C-labeled at residues $K^{16}L^{17}V^{18}$, suggested that this region of the peptide is flexible, likely involved in a loop between two b-strands.

Both pure Aβ_{1-42} and pEAβ_{3-42} with ^{13}C uniformly labeled at $K^{16}L^{17}V^{18}$ exhibit broad NMR linewidth (3–4 ppm) indicating highly inhomogeneous and polymorphic conformation. Similar broad resonances were observed with $V^{36}G^{37}G^{38}V^{39}$ labeled pure Aβ_{1-42} and pEAβ_{3-42} samples. Meanwhile, staff observed the downshift of C´ and C_α peaks and upshift of C_β for both peptides ^{13}C labeled at $V^{36}G^{37}G^{38}V^{39}$ in their 2D dipolar assisted rotational resonance (DARR) ^{13}C–^{13}C spectra. This represents β–sheet conformation in the labeled region. In addition, the linewidth reduced to 2 ppm or less, suggesting that this region assumes uniform conformation after reconstitution in lipid membrane. Using 1D and 2D ^{13}C-^{13}C DARR spectra of segmentally labeled and uniformly labeled Ab peptides, residue type assignments of many amino acid residues have been accomplished, which will produce atomic resolution structure of the peptide.

Follow On Funding: Florida Department of Health - $249,999 (pending); National Institute of Health - $1,786,834 (pending).

Collaborations: None at the time of reporting.

Journals: Tatulian, S.A. and Kandel, N. Membrane Pore Formation by Peptides Studied by Fluorescence Techniques. *Methods Mol. Biol.* 2003, 449-464 (2019) doi: 10.1007/978-1-4939-9512-7_19; PMID:31218629.

Kandel, N., Matos, O.M., and Tatulian, S.A. Structure of amyloid β_{25-35} in lipid environment and cholesterol-dependent membrane pore formation. *Sci. Rep.* 9(1):2689 (2019) doi: 10.1038/s41598-019-38749-7; PMID:30804528

Patents: None at the time of reporting.

APPENDIX E
FISCAL YEAR 2018-2019 COMPLETED GRANTS
(Funding Year 2017-2018)

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
8AZ01	Ave Maria University	Barbosa, Antonio	$100,000	$ 84,838.95	$ 15,461.05	02/07/2018	02/28/2019	No	Yes	No
8AZ10	Mayo Clinic Jacksonville	Ebbert, Mark	$100,000	$100,000		02/13/2018	02/28/2019	No	Yes	Yes
8AZ21	University of Miami	Curiel, Rosie	$89,304	$88,203.65	1,100.35	02/28/2019	02/28/2019	No	Yes	No

COMPLETED GRANTS FISCAL YEAR 2018-2019
(Funding Year 2017-2018)

1. **Grant #8AZ01:** Inhibiting Alzheimer's Disease by Modulating a Key Player in Plaque and Tangle Formation, SIRT1, by Regulating the Formation of Nicotinamide Metabolites

   Principal Investigator: Antonio Barbosa, PhD

   Organization: Ave Maria University

   Progress Report: The synthesis of six SIRT1 agonists representing the varied heterocyclic core replacements has been successfully completed. Progress toward the synthesis of compounds targeting a potential hydrogen bond with the carbonyl group of proline-155 SIRT1 to yield a potential boost in potency and selectivity has begun and been met with some challenges but should be completed in the coming months. \

   The compounds synthesized above were then tested in a SIRT1 assay with the Boc-Lys (Ac)-AMC substrate. Two compounds showed clear activation of SIRT1. Additionally, other compounds were identified that strongly inhibit SIRT1 activity. These compounds will be followed up with the synthesis of novel agonists and antagonists of SIRT1.

   Work to develop an additional SIRT1 assay has progressed. This continuous coupled enzyme assay has already been described by other labs but requires acetylated peptide and nicotinamidase enzyme. The known SIRT1 substrate peptide that corresponds to an acetylated portion of CRABPII has been successfully synthesized and nicotinamidase was successfully cloned, expressed, purified, and assayed.

   SIRT1 and NNMT were successfully expressed and purified in the lab, and activity for both enzymes was demonstrated. Using the assays described with the Boc-Lys (Ac)-AMC substrate, Me-NAM has been demonstrated to act as an allosteric regulator of SIRT1. The assays demonstrate that Me-NAM is NOT an inhibitor of SIRT1. This suggests that NNMT is able to influence the activity of SIRT1 by metabolizing an inhibitor and ameliorating its negative regulation.

   Progress was made towards the synthesis of Aldehyde oxidase (AOX). AOX genes were successfully cloned from human and mice. Additionally, an aldehyde oxidase expression construct was obtained from a collaborator as well as e. coli expression strains that are designed to optimize the production of the molybdopterin cofactor. Expression studies and SDS-PAGE gels revealed what appears to be successful AOX overexpression. However, after purification, protein with the desired molecular weight was not obtained, and little to no activity was detectable. Numerous attempts were made to try to overcome these protein production issues, and eventually the focus shifted to the design of a new Me-NAM detection assay.

   Two in vitro assays of situin activity in yeast, a longevity assay and a transcriptional inhibition assay have been evaluated in order to better assess the putative SIRT1

activators. The longevity assay proved not to be reliable enough for screening biological activity of putative SIRT1 activators. The Transcriptional inhibition assay has been developed and is in the process of being evaluated.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Mode of Action of Granulocyte-colony Stimulating Factor (G-CSF) as a Novel Therapy for Stroke in a Mouse Model. (Journal of Biomedical Science. (In review).

Patents: None at the time of reporting.

2. **Grant #8AZ10:** Identifying Drug Targets using Long-Read Sequencing In Alzheimer's Diseased and Control Brain Tissue

   Principal Investigator: Mark T. W. Ebbert, PhD

   Organization: Mayo Clinic Jacksonville

   Progress Report: Research staff were able to accomplish many important items, including two high-quality research publications in *Moleculary Neurodegeneration*, and staff learned that the second publication was accepted to *Genome Biology*. Researchers successfully generated preliminary data to obtain a full Florida Ed & Ethel Moore Alzheimer's research grant, and to submit an NIH R01 grant. Staff are also preparing an additional NIH R01 grant based on results from the second paper. Preliminary data included targeted long-read sequencing data for both DNA and RNA. The funds from the Florida Ed & Ethel Moore grant were critical to generating these data. Staff are confident that it will lead to additional funding for this research. Because of the funds in this grant, staff were also able to hire a new bioinformatics technician in the lab, and support partial wages for a laboratory technician. Research staff have identified several structural DNA mutations that may be involved in Alzheimer's disease. Staff are pursuing additional federal funds to test these mutations in a larger cohort.

   Follow On Funding: Muscular Dystrophy Association - $300,000 (funded); Bright Focus - $300,000 (not funded); Florida Department of Health - $250,000 (funded); National Institutes of Health, National Institute on Aging - $2,000,000 (pending).

   Collaborations: Alzheimer's Disease Sequencing Project, Alzheimer's Disease Neuroimaging Initiative, CReATe Consortium, Target ALS, and Banner Health

   Journals: Mark T.W. Ebbert, Stefan L. Farrugia, Jonathon P. Sens, Karen Jansen-West, Tania F. Gendron, Mercedes Prudencio, Ian J. McLaughlin, Brett Bowman, Matthew Seetin, Marley DeJesus-Hernandez, Jazmyne Jackson, Patricia H. Brown, Dennis W. Dickson, Marka van Blitterswijk, Rosa Rademakers, Leonard Petrucelli, and John D. Fryer; *Long-read sequencing across the C9orf72 'GGGGCC' repeat expansion:*

implications for clinical use and genetic discovery efforts in human disease; Molecular Neurodegeneration, August 2018.

Mark T.W. Ebbert, Tanner D. Jensen, Karen Jansen-West, Jonathon P. Sens, Joseph S. Reddy, Perry G. Ridge, John S. K. Kauwe, Veronique Belzil, Luc Pregent, Minerva M. Carrasquillo, Dirk Keene, Eric Larson, Paul Crane, Yan w. Asmann, Nilufer Ertekin-Taner, Steven G. Younkin, Owen A. Ross, Rosa Rademakers, Leonard Petrucelli, John D. Fryer; Systematic analysis of dark and camouflaged genes reveals disease-relevant genes hiding in plain sight; Genome Biology; April 2019.

Patents: None at the time of reporting.

3. **Grant #8AZ21:** Postdoctoral Fellowship In Neuropsychology

   Principal Investigator: Rosie E. Curiel, PsyD

   Organization: University of Miami Miller School of Medicine

   Progress Report: Dr. Daema Piña, is the postdoctoral trainee focused on working with Dr. Curiel on the Study Precision-Based Computerized Assessment for the Detection of Mild Cognitive Impairment in Older Adults. This is a NIH-funded longitudinal study on aging and cognition, that began enrollment in July, 2018. Dr. Piña assisted on the development of the study protocol and the memory evaluation tools including the neuropsychological tests. Dr. Piña also actively assisted in training the staff members and the doctoral-level students that participated in the implementation of the research study. Dr. Piña implemented a bi-weekly Didactics Training Series for the doctoral-level students that are collaborating in the research study and clinical services at the Center for Cognitive Neuroscience and Aging. At least eleven students and three staff members attended the training series.

   During the postdoctoral training, Dr. Piña supported with beta testing and evaluated over 50 individuals in the clinical research program. Dr. Pina's assistance with piloting and helping to refine the experimental measures was a new experience and she was exposed to understanding almost every operational aspect of the project, from attending recruitment events to data collection and data entry.

   Dr. Piña assisted with the submission of multiple grant applications including a submission to the Ed and Ethel Moore AD research program with Co-Mentor, Dr. Loewenstein and thus received additional training on grant writing, and conceptualizing study methodology. Moreover, Dr. Piña continued work with Dr. Loewenstein in obtaining follow-up MRIs for the ongoing R01 study and received training on writing manuscripts.

   Dr. Piña transitioned to become an Assistant Professor/Clinical Neuropsychologist for the Department of Psychiatry and Behavioral Sciences at the University of Miami – Miller School of Medicine. Dr. Pina will continue to evaluate patients with neurodegenerations.

   Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Rosie E. Curiel, Elizabeth A. Crocco, Arlene Raffo, Salvador M. Guinjoan, Charles Nemeroff, Ailyn Penate, Daema Piña and David A Loewenstein; *Failure to Recover from Proactive Semantic Interference Differentiates Amnestic Mild Cognitive Impairment and PreMCI from Normal Aging after Adjusting for Initial Learning Ability*; Scientific Research, June 2018.

Rosie E. Curiel, PsyD, David A. Loewenstein, PhD, Monica Rosselli, PhD, Daema Piña, PsyD.; *Cross-Cultural Applicability of a Cognitive Stress Test for Preclinical Alzheimer's Disease*, Alzheimer's and Dementia (under review).

Patents: None at the time of reporting.

APPENDIX F
FISCAL YEAR 2018-2019 COMPLETED GRANTS
(Funding Year 2016-2017)

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
7AZ01	University of Florida	Smith, Glenn	$450,000	$422,942.42	$ 27,057.58	02/01/2017	03/31/2019	No	Yes	Yes
7AZ02	Florida International Universtiy	Burke, Shanna	$99,994	$99,994		02/01/2017	03/31/2019	No	Yes	Yes
7AZ03	University of South Florida	Kang, David	$ 250,000	$ 240,742	$ 9,258	1/26/2017	3/31/2019	No	Yes	No
7AZ04	University of Miami	Rotundo, Richard	$ 250,000	$ 222,224	$ 27,776	2/01/2017	3/31/2019	No	No	No
7AZ05	Mayo Clinic Jacksonville	McLean, Pamela	$250,000	$238,204.11	$11,795.89	02/02/2017	03/31/2019	No	No	No
7AZ06	University of Florida	Bizon, Jennifer	$ 100,000	$98,866.84	$ 1,113.16	01/25/2017	03/31/2019	No	No	Yes
7AZ12	University of Florida	Rincon-Limas, Diego	$ 249,998	$ 222,224	$ 27,774	2/06/2017	9/30/2019	No	No	Yes
7AZ13	University of South Florida	Gulick, Danielle	$100,000	$93,694.88	6,305.12	02/14/2017	03/31/2019	No	No	No
7AZ14	University of Miami	Curiel, Rosie	$ 249,846	$ 216,505.27	$ 33,340.73	2/23/2017	9/30/2019	No	Yes	Yes
7AZ17	Mayo Clinic Jacksonville	Ertekin-Taner, Niluter	$308,807	$297,519.69	11,287.31	02/05/2017	03/31/2019	No	Yes	No
7AZ18	University of Miami	Loewenstein, David	$ 249,980	$ 192,832	$ 57,148	2/27/2017	9/30/2019	No	Yes	Yes
7AZ19	University of Florida	Levites, Yona	$ 250,000	$ 222,224	$ 27,776	2/01/2017	9/30/2019	No	Yes	Yes
7AZ20	University of Miami	Cukier, Holly	$ 250,000	$ 222,224	$ 27,776	2/01/2017	9/30/2019	No	No	No

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
7AZ23	University of South Florida	Cheng, Feng	$ 98,449	$ 87,504	$ 10,945	2/01/2017	9/30/2019	No	Yes	Yes
7AZ24	University of South Florida	Gamsby, Joshua	$ 100,000	$ 96,403.68	$ 3,596.32	2/01/2017	3/31/2019	No	Yes	Yes
7AZ25	University of Florida	Giasson, Benoit	$ 250,000	$ 224,330	$ 25,670	2/01/2017	9/30/2019	No	Yes	No
7AZ28	Florida Atlantic University	Wiese, Lisa	$ 95,133	$ 93,641.90	$ 1,491.10	5/15/2017	6/30/2019	No	Yes	Yes

COMPLETED GRANTS FISCAL YEAR 2018-2019
(Funding Year 2016-2017)

1. **Grant #7AZ01:** Physical Exercise and Cognitive Engagement Outcomes for Mild Neurocognitive Disorder (PEACEOFMND)

   Principal Investigator: Glenn Smith, PhD

   Organization: University of Florida

   Progress Report: This project delivered on its primary aim of establishing a collaboration among the Memory Disorders Centers at Tallahassee Memorial Healthcare (TMH), Mayo Jacksonville, and the University of Florida (UF). And while a clinical research intervention program for Mild Cognitive Impairment (MCI) previously existed at Mayo this funding enabled the establishment of comparable programs at TMH and UF. Progress was slowed somewhat by the turnover of the TMH site PI in the summer of 2018 and the study coordinator at Mayo Jacksonville that fall. Nevertheless, not only has the program made significant progress towards meeting its recruitment target, but staff have created a common database that will be incorporated into a larger registry of patients from the Mayo HABIT program, which is a large dataset examining the comparative effectiveness of different behavioral interventions for MCI.

   Researchers have received subsequent funding from the Ed and Ethel Moore program 9AZ15 *Association of PET amyloid status with cognitive and functional outcomes of behavioral interventions in Mild Cognitive Impairment.* This grant will permit us to recruit participants from 7AZ01 to return for a 18 to 24 month follow-up and to obtain amyloid PET scans on returning participants. The study will permit us to examine whether outcomes in that study associate with amyloid status.

   Follow On Funding: Florida Department of Health - $237,500

   Collaborations: Established a collaboration among the Memory Disorders Centers at Tallahassee Memorial Healthcare (TMH), Mayo Jacksonville, and the University of Florida (UF). A common database was setup and personnel trained at all sites to criterion for recruitment and intervention delivery by having TMH personnel travel to both Mayo and UF and having UF personnel attend TMH's inaugural session. Weekly phone calls were maintained for the first year of the project and bimonthly phone calls thereafter to assure coordination and consistency across sites.

   Journals: O'Shea, D. M., Tanner J., De Wit, L., DeFeis, B., Mejia, A., Amofa, Chandler M. & Smith G. (2019). Prediction of Response to Behavioral Interventions with MRI-Based Hippocampal Subfields in Mild Cognitive Impairment: Preliminary Findings. Poster presented at 47th annual International Neuropsychology Society (INS) meeting, New York, NY.

   Patents: None at the time of reporting.

2. **Grant #7AZ02:** Demographic, Neuropsychological and Functional Classification, Risk Factors, and Progression Rates of Individuals Diagnosed as “Impaired Not MCI” in the National Alzheimer’s Coordinating Center Database Using Algorithmic Diagnosis

   Principal Investigator: Shanna L. Burke, PhD

   Organization: Florida International University

   Progress Report: The National Alzheimer’s Coordinating Center (NACC) Uniform Data set (UDS) was utilized as the algorithm training dataset as it contained data from 35 past and present Alzheimer’s Disease Centers from 2005 to 2017, 118,341 participant visits and 35,183 unique participants. To determine variable inputs, staff tested the ability of Clinical Dementia Rating Sum of Boxes (CDRsb) scores to distinguish between adjacent cognitive impairment diagnoses versus four different combinations of neuropsychological tests. Research staff determined the optimal cut-off point of CDRsb that maximized the sensitivity and specificity of the Receiver Operating Characteristic (ROC) curve and applied the cut-off point to the validation sample to obtain the predicted diagnoses. Using the information derived from the ROC analyses, research staff then codified the algorithm based on the Duara et al., (2010) consensus diagnosis variables from the NACC data set. Staff called the resulting algorithm the (eAlgDx). In all, there are 294 possible permutations of the eAlgDx algorithm.

   The optimal cut off point maximizing the sensitivity and specificity was normal vs MCI:0.25 and for MCI vs Alzheimer’s disease (AD): 3.25. This means CDRsb score of 0 is equivalent to normal cognition, and a CDRsb score between 0.5 and 3 is indicative of MCI, and a CDRsb score between 3.5 and 18 indicates AD. The level of agreement between the original NACC diagnoses and the eAlgDx was low which was expected. Staff anticipated that the algorithm would be more sensitive to the newly established cutoffs and would be able to further subdivide the cases that were normal, impaired not MCI, MCI, and demented as indicated in the original dataset. Staff repeated the ROC analyses using the eAlgDx to explore how well it distinguishes the adjacent diagnostic categories, which was previously conducted in the raw data. For example, staff found that of the 78,401 visits previously classified as impaired not MCI, only 5,636 cases remained as impaired not MCI, and other were reclassified in a more precise manner. The eAlgDx algorithm was able to reclassify 72,765 visits as dementia (31,073), MCI (20,498) or normal (21,194) using the newly established cutoffs. Furthermore, staff analyzed 7,228 visit results, which were originally classified as MCI. The new sensitivity of the eAlgDx reclassified these into 169 occurrences of dementia, 3,180 occurrences of MCI, and 3,879 occurrences of normal cognitive status.

   In aim 2, research staff compared the importance of demographic and psychosocial factors to the new eAlgDx as compared to the original diagnoses. The most important variables were determined to be: anxiety, APOE e4, depression in the last 2 years, education, sleep disturbance, age, sex, marital status, depression more than 2 years ago, first degree family member with cognitive impairment, stroke, alcohol abuse – clinically significant occurring over a 12-month period manifested in one of the following

areas: work, driving, legal, or social domains, race, other cardiovascular disease, total number of years smoking cigarettes, Hispanicity, hypertension, hypercholesterolemia, heart attack/cardiac arrest history, primary language, diabetes, and history of traumatic brain injury (TBI). Finally, for aim 3, staff compared rates of progression to an increasingly severe cognitive status when using the original diagnosis versus the eAlgDx as well as how the aforementioned demographic and psychosocial factors modulate the risk profiles.

The first manuscript, detailing the results of aim 1, is nearly complete, and research staff expect this to be under review by July 1, 2019. The second manuscript is 50% complete, and staff expect this to be under review by August 15, 2019. Staff presented the team's findings for aims 1 and 2 at the Florida Health Alzheimer's Disease Research and Awareness Symposium, Orlando, FL, on June 8, 2018. In August 2018, the PI met with investigators in Chicago, IL, to discuss risk and protective factors for longevity and neurodegeneration, which could be included in the algorithm in aim 2 (BMI, caloric intake, cardiovascular risk factors, and genetics). In November 2018, staff presented the team's results in an oral presentation at the Gerontological Society of America scientific meeting in Boston, MA. The code was released on GitHub in November 2018 (https://github.com/senrabc/dxster_nacc/releases).

Follow On Funding: Department of Defense - $640,157.21 (pending); Florida Department of Health - $249,239.69 (pending).

Collaborations: The key personnel and scientific programs and the project have benefited from the collaboration between UF and FIU and consulting from Dr. Loewenstein of the University of Miami.

Journals: Burke, S., Barnes, C., Hanson, K., Hu, T., Duara, R., Loewenstein, D.A. *A deterministic approach to algorithmic consensus diagnosis: Computerizing the conversation*. (will submit by July 1, 2019)

Burke, S., Barnes, C., Hanon, K., Hu, T., Wu, W., Gonzalez, I., Naseh, M., Grudzein, A., Duara, R., Loewenstein, D.A. Manuscript 2. (will submit by August 15, 2019)

Patents: None at the time of reporting.

3. **Grant #7AZ03:** Structure Activity Characterization of Novel Slingshot Inhibitors

Principal Investigator: David Kang, PhD

Organization: University of South Florida

Progress Report: Research staff synthesized more than 10 methylated derivatives of C2, three of which showed significantly improved inhibitory activity against SSH1 (Ben-131>Ben-132>C2). The compounds similarly showed improved inhibition of tau phosphorylation in primary neurons and cultured cells. To further optimize these compounds, staff attempted to add additional methyl groups to the improved compounds, which proved difficult. Hence, staff are taking a two-pronged approach to

improving the lead series – exploring the hydrophobic pocket staff identified at the start of this work, and use of carboxylate isosteres in the search for improved physicochemical properties without loss of SSH1 activity. On the crystallographic front, staff have succeeded in crystalizing apo SSH2 at 2 angstrom resolution. While staff screened numerous crystallization conditions for protein complexes with C1 and C2, co-crystallization of compounds with SSH1 proved difficult. Staff have now devised a method to test direct in vitro binding of compounds to SSH1 using surface plasmon resonance (SPR), which should reveal direct binding affinities and kinetics. Using SPR experiments, staff have successfully determined the Kd values for C2, Ben-131, Ben-132, and Ben-133 compounds, which were in the low micromolar range. Binding compounds to SSH1 on SPR chip beyond 125 micromolar range resulted in aggregation of the compounds on the chip. Staff have successfully purified multiple batches of recombinant proteins SSH1, PRL, and PTEN phosphatases using the sf9 baculovirus insect sell system. These batches of proteins, unlike those purchased from commercial sources, proved to yield high phosphatase activity. Multiple experiments showed that C1 and C2 compounds have a 3-10x higher potency toward SSH1 than PRL and PTEN, which researchers believe could be further improved via medicinal chemistry optimization. Staff have now determined the ADME properties of the C@ derivatives using in vitro cell-based assays.

Follow On Funding: None at the time of reporting.

Collaborations: This project is collaborating with USF Health: Kang, Chen, Leahy labs.

Journals: Woo JA*, Liu T, Fang CC, Cazzaro, S, Kee T, LePochat P, Yrigoin K, Penn C, Zhao X, Wang X, Liggett SB, Kang DE*. Commun Biol. 2019 Mar 22; 2:112. Doi: 10.1038/s42003-019-0359-9. ECollection 2019. *corresponding authors.

Dual role of Cofilin in APP trafficking and Amyloid β Clearance. 2019. Liu, T., Woo, J.A., Yan, Y., Kang, D.E. FASEB J. In minor revision.

Kang, D.E., Woo, J.A. (2019). Cofilin, a master node regulating cytoskeletal pathogenesis of Alzheimer’s disease. J Alzheimers Dis, In minor revision.

Patents: None at the time of reporting.

4. **Grant #7AZ04:** Enhanced Acetylcholinesterase Expression Induced by Donepezil and Galantamine

   Principal Investigator: Richard L. Rotundo, PhD

   Organization: University of Miami Miller School of Medicine

   Progress Report: Acetylcholinesterase (AChE) is the enzyme that terminates neurotransmission at all cholinergic synapses in the central and peripheral nervous systems. These are the synapses that initially degenerate in Alzheimer's disease and are in large part responsible for the loss of memory. The initial discovery from this lab that formed the basis for this project was that the small molecule inhibitors of AChE, the

most important drugs used to treat Alzheimer's disease (AD) patients, could also act as molecular chaperones to enhance protein folding, thereby increasing AChE expression in the brain. The original Aims for the first year were to test a series of AChE inhibitors from different classes (with different biochemical properties) to determine which types could enhance AChE folding. Research staff found that only the AChE inhibitors from the class called Active Site Directed Inhibitors (ASDIs) directly affect AChE folding by stabilizing the folding intermediates. Unfortunately, these are the major inhibitors used to treat Alzheimer's patients. Staff then set about testing combinations of ASDI inhibitors with carbamate-type inhibitors (like Rivastigmine) to determine a ratio that would inhibit AChE by at least 50% yet at the same time minimize or eliminate the enhanced protein folding effect. These experiments were all done with tissue cultured cells expressing murine AChE. Staff developed a combination that in principle should inhibit AChE catalytic activity while at the same time minimize excess AChE folding. Staff accomplished the first two Specific Aims focusing on studies showing that the ASDIs had major effects on AChE folding both in culture and in vivo using a mouse model. The third Specific Aim consisted of testing the hypothesis that these ASDI inhibitors would impair memory and learning using the same mouse model and behavioral testing in a radial arm maze, whereas other types of inhibitors such as the carbamate Rivastigmine would not. This hypothesis was not supported by the results, where all mice were affected the same regardless of treatment. It is not clear why the differences did not appear, and additional experiments will be needed to understand these results. The experiments from Aims I and II are strong and are being written up 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.

5. **Grant #7AZ05:** How Does Alpha-Synuclein Contribute to Tau Dysfunction in Alzheimer’s Disease?

   Principal Investigator: Pamela McLean, PhD

   Organization: Mayo Clinic Jacksonville

   Progress Report: In this application, research staff proposed to determine if there was previously undetected αsyn and tau in AD brain and use mouse models of tau aggregation to investigate if αsyn contributes to tau dysfunction and the progression of disease in Alzheimer’s disease and related neurodegenerative diseases. Aim 1 of the proposed study was completed, with data supporting the fact that previously undetected αsyn oligomers are not a significant pathologic finding in human post-mortem AD brain. For Aim 2, research staff successfully optimized the proximity ligation assay to detect αsyn-tau interactions in human brain precluding the conclusion that these interactions are absent versus the possibility that the PLA assay did not work efficiently on human

brain. For Aim 3, staff successfully generated a three-month cohort of αsyn transgenic and non-transgenic mice overexpressing tau throughout the brain via AAV injection at postnatal day 0. Characterization of the three-month cohort revealed no significant differences in pathology with a trend towards increased fibrillar tau in αsyn transgenic animals. These data are currently being confirmed in the six-month cohort of mice which was not able to be completed by the end of the 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.

6. **Grant #7AZ06:** Impact of Perirhinal Cortical Tau Pathology on Pre-Clinical Cognitive Decline

   Principal Investigator: Jennifer Bizon, PhD

   Organization: University of Florida

   Progress Report: During the two years of this pilot funding, the only significant change was that the postdoctoral researcher on this project, Dr. Sarah A. Johnson, received a K99 grant. As a result, Dr. Johnson shifted her focus to working on her own grant and researchers needed to hire a research technician to complete the aims of this project. This did not impact the research, and in the past two years staff have completed the primary goals of this pilot study. In addition to working towards the major goal of developing a new model of tauopathy that accounts for interactions with advancing age, staff have also used these preliminary data to successfully obtain National Institutes of Health/National Institute on Aging grants that have brought additional money into the state of Florida.

   The significant scientific accomplishments are summarized below.

   Validated that viral vector technology with AAV1 containing either human wild-type tau or the P301L mutation could produce robust AT8 labeling in the perirhinal cortex of young and aged rats. AT8 is an antibody that identifies hyperphosphorylated tau.

   Using AAV1-GFP, staff showed that viral transfection was similar in aged and young rats.

   Demonstrated that total tau expression and phosphorylated tau were increased in rats that received AAV1-wtTau, but not the GFP control animals.

   Demonstrated labeling with the Alz50 antibody in AAV1-wtTau animals, but not with AAV1-GFP controls.

   Demonstrated that Alz50 is an indicator of more advanced conformational changes in tau (that is, pre-tangles).

Upgraded experimental controls for quantifying olfactory discrimination, which is hypothesized to be a behavioral biomarker of early tau pathology in the perirhinal cortex.

Demonstrated that AAV1-mediated perirhinal cortical tau pathology spread to the hippocampus. This was evident in slices and cleared brain tissue.

Validated a novel touch-screen based behavioral paradigm for measuring perceptual discrimination. This behavior is exquisitely sensitive to perirhinal cortical dysfunction.

Demonstrated a clear perceptual gradient in the ability to discriminate between two visual stimulation as a function of stimulus overlap. This was a critical step for developing a new behavioral biomarker for early tau pathology.

Demonstrated that the proportion of cells in the perirhinal cortex that were both GFP and AT8-positive are higher in the aged compared to young rats. This suggests that age accelerates tau pathology progression.

Demonstrated an increase in cell size among neurons that were positive for AT8. This could reflect neuron hypertrophy, which has been reported in the early stages (asymptomatic Alzheimer's disease).

The research team completed an extensive Western body panel to quantify phosphorylated tau levels in the perirhinal cortex of young and aged rats. Notably, for the CP27 antibody (which reacts to human tau and does not cross-react with rat tau) that was a band of the correct size in the aged AAV-hWtTau rats. This band was not present in the young animals (control or tau) or the aged controls rats. This band is human tau as it is recognized by CP27 which does not cross-react with rat tau. Moreover, in many aged AAV-hWtTau animals, the same band is detected with a 7F2 antibody which recognizes pSer202-pThr205 (also recognized with AT8 antibody). Together these data suggest that human tau delivered to aged rats is becoming hyperphosphorylated in area 35 (the site of delivery) two months after AAV delivery. This did not occur to the same extent in young rats.

When compared the two- to six-month survival time points, the research staff observed that 6 months after wtTau expression in the perirhinal cortex, there is regression of fibers that is not evident in the two month group. This could be indicative of more advanced pathology.

Research staff have already been successful at acquiring follow-on funding. First, an R21 (NIH/NIA R21AG058240) grant was awarded to focus on conducting an extensive biochemical analysis of young and aged rats with human wt-tau in the perirhinal cortex, as well as a longitudinal behavioral assessment. These goals were not included in the current pilot grant, and future research will characterize tau in the insoluble fractions from rats that have received AAV1-wtTau. Staff will also expand the biochemical analysis to include additional antibodies, recognizing different features of pathological tau and will expand the cohort to include young and aged rats delivered a mutant version of tau (P301L) at the same two-month survival time after surgery. Finally, research staff will

process the hippocampus of these same animals to quantify the extent to which tau pathology is occurring in monosynaptic sites outside of the site of AAV-WtTau delivery.

Since staff have observed that tau pathology spreads to brain regions that are beyond the AAV injection site, including the amygdala, researchers have obtained an R01 (NIH/NIA R01AG060778) grant that will determine the impact of tau pathology in the amygdala on deliberative decision-making behavior. The amygdala is critical for decision-making, and shows biochemical alterations with age even in the absence of pathology. Thus, a future avenue of research will be to examine the interaction between age and tau pathology in the context of an intertemporal choice task, which measures the extent that an animal prefers a small immediate reward over a large delayed reward.

A final future direction will be to examine how metabolic function interacts with the development of tau pathology. A recent award of an R01 grant has been obtained to pursue this work (NIH/NIA R01AG060977). There is a strong relationship between metabolic dysfunction and risk of Alzheimer's disease. To date, however, anti-diabetic drugs have been insufficient for improving cognition and delaying the rate of disease progression. Critically, PET scans of Alzheimer's disease patients show severe deficits in glucose uptake, but normal uptake of ketone bodies. Recently, low carbohydrate diets with medium-chain triglyceride supplementation have been used to attenuate cognitive deficits in patients with Alzheimer's disease and mild cognitive impairment, suggesting that nutritional ketosis may be a metabolic state that can improve brain function in Alzheimer's disease. In line with this idea, studies in transgenic mouse models of Alzheimer's disease have shown that a ketogenic diet initiated in young adults can slow the progression of pathology. Importantly, the disease-modifying capacity of a ketogenic diet has not been evaluated in aged animals. Since the pilot data collected under this DOH grant clearly show that aging accelerates the progression of tau pathology, is it critical to evaluate metabolic interventions for Alzheimer's disease in aged animals; this will be the focus of future research plans.

Follow On Funding: National Institute of Health/National Institute on Aging - $275,000, $415,000, $3 million, and $2 million.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

7. **Grant #7AZ12:** Large-Scale Identification of Genes that Suppress Concurrent Abeta42 and Tau Pathology in Vivo

   Principal Investigator: Diego E. Rincon-Limas, M.Sc., PhD

   Organization: University of Florida

   Progress Report: Alzheimer's disease (AD) is a complex degenerative brain disorder characterized by memory loss, cognitive decline, and neuronal cell death, and is the

most prevalent cause of dementia among the elderly. Indeed, AD affects an estimated 5.8 million individuals in the US and 47 million worldwide. Sadly, there is no way to cure AD or to stop or delay its progression. It is clear that the Aβ42 peptide and tau are key molecular culprits of the disease because the AD brain displays extracellular deposits of Aβ42, known as senile plaques, and intracellular neurofibrillary tangles (NFTs) carrying abnormal forms of the protein tau. Recent studies demonstrate that synergistic interactions between Aβ42 and tau exacerbate the pathogenesis of AD, suggesting that both proteins should be addressed simultaneously to achieve more effective therapies. Unfortunately, it is unknown how Aβ42 and tau induce their synchronized toxicity and which cellular factors could be targeted to block their pathological interactions.

The major goal of this project was to decode the pathological crosstalk between Aβ42 and tau, and their interacting mechanisms, and to identify new genes that may serve as therapeutic targets. To that end, the Principal Investigator generated a new fruit fly model of AD by producing human Aβ42 and tau in the fly brain. These “humanized” flies exhibit Aβ42 aggregation, tangle-like deposition of tau, and robust neuronal loss. Given the short life-cycle of the fruit fly (~10 days), this model provided a unique discovery platform to screen thousands of genes and look for those that have the ability to modify the course of AD pathology in flies.

During this reporting period, the research team tested almost 4,000 different genes and identified 31 suppressors and 92 enhancers of the pathology induced by Aβ42 and tau in flies. Staff also conducted an extensive bioinformatics analysis of the identified genes to understand their cellular functions and to define how they interact with each other. Staff found that most suppressors have functions associated with protein modification or cleavage, cell metabolism, translation, transcription, chromatin modulation, and transport to name a few. Of note, staff identified few genes that were previously linked to AD pathology (such as cellular stress factors or chaperones), but most of them are not previously known to be associated with the initiation or progression of the disease. Taken together, all these genetic targets represent a new opportunity to understand the molecular complexity underlaying coexistent Aβ42 and tau pathologies. Staff strongly believe that the results of this project will have important implications for the development of new therapeutic strategies and, therefore, will directly benefit the 540,000 Floridians affected with this devastating disease.

Follow On Funding: National Institute of Health - $412,211 (pending).

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

8. **Grant #7AZ13:** CK1 Delta Inhibition to Reduce Sundowning in Alzheimer's Disease

   Principal Investigator: Danielle Gulick, PhD

Organization: University of South Florida

Progress Report: Research staff have demonstrated that PF-670462, a small molecular inhibitor of CK 1d/e alters circadian rhythmicity, improves affect and cognition, and decreases amyloid load in the APP-PS1 mouse. Together, these findings support the potential efficacy of chronotherapeutic interventions in Alzheimer's disease.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

9. **Grant #7AZ14:** Precision-Based Assessment for Detection of MCI in Older Adults

Principal Investigator: Rosie Curiel, PsyD

Organization: University of Miami

Progress Report: The AIM study (Assessment Innovations in Mild Cognitive Impairment) examined individuals over the age of 60 in south Florida who are of normal cognition or at risk for developing Alzheimer's disease and related disorders. Research staff established collaborations with the University of Miami Department of Computer Sciences, Radiology, and Neurology to ensure that staff meet the project aims. The computerized cognitive stress tests were developed and deployed in English and Spanish to address the disparity related to the early identification of Hispanic elders in Florida, which is a critical priority. For each participant, staff obtained relevant health information, conducted a neuropsychological assessment using traditional measures, obtained data on these experimental measures, and collected biological markers such as brain imaging and blood for APOE genotyping. Consensus Diagnoses for each case were conducted with the study team, including PI Rosie Curiel, PsyD, Co-I David Loewenstein, PhD, Marcela Kitaigorodsky, PsyD, and the Chief of Geriatric Psychiatry and the Director of the state-funded memory disorders center, Dr. Elizabeth Crocco, who rendered highly-skilled diagnostic consensuses.

The study team exceeded the recruitment goal. A total of 132 older adults were enrolled in the study; 60 of them were primarily Spanish speakers and 72 of were primarily English speakers. 65% of the participants enrolled were minorities. All the enrolled participants completed the novel computerized tests. Additionally, 77 of them completed a re-test session to validate the computerized testing measures and 82 had the study Magnetic Resonance Imaging (MRI). The group of investigators is now analyzing the collected data and preparing manuscripts regarding the study results for publication purposes.

Pilot data obtained during this study facilitated an application to the National Institute of Health/National Institute on Aging and PI, Dr. Curiel, received a five-year longitudinal

RO1 grant to continue this research. This research study has been a unique opportunity to study the earliest preclinical manifestations of Alzheimer's disease (AD) by optimizing assessment methods and relating these to biological markers of atrophy on MRI signature regions. Moreover, the study focused on recruiting Hispanic individuals, who represent an ever-growing yet understudied group. This work has a critical health impact on older adults within the state since staff provided state-of-the art methods to improve early detection of AD. Although there is currently a lack of effective treatments for the underlying causes of AD (current methods only alleviate symptoms for a limited period of time), this proposed effort is akin to previous work in many forms of cancer in which earlier diagnosis led to more effective treatments and increased quality of life.

Follow On Funding: FSDH - $84,301; National Institute on Aging - $3 million.

Collaborations: Nova Southeastern University, College of Psychology. Doctoral students from the Clinical Psychology Program assisted in the research activities as part of their practicum experience program.

Carlos Albizu University. Doctoral students from the Clinical Psychology Program assisted in the research activities as part of their practicum experience program.

The Doctoral-level students from the two institutions listed above are completing a clinical research practicum at our site and are involved with the study as psychometrists. They are also encouraged to work with the PI to analyze the study data to present scientific findings at scientific meetings. Institutional collaborations are strong to carry out the study aims with the Departments of Radiology, Neurology, and Computer Sciences.

Journals: Rosie E. Curiel Cid, David A. Loewenstein, *A cognitive stress test for prodromal Alzheimer's disease: Multiethnic generalizability*, ELSEVIER, 11/2019.

Noam Alperin, Rosie Curiel Cid and David Loewenstein, *Effect of sleep quality on amnestic mild cognitive impairment vulnerable brain regions in cognitively normal elderly individuals*, Sleep Research Society, 01/2019.

Patents: None at the time of reporting.

10. **Grant #7AZ17:** Florida Consortium for African-American Alzheimer's Disease Studies (FCA3DS)

    Principal Investigator: Nilufer Ertekin-Taner, MD, PhD

    Organization: Mayo Clinic Jacksonville

    Progress Report: During this reporting period research staff have accomplished the following:

    Aim 1:

Obtained modification to the IRB protocol (IRB#: 15-000189; IRB Modification #: 15-000189-04) which enabled staff to expand the studies to include the proposed work in this grant.

Obtained approval to utilize the data and/or samples from the following studies, allowing staff to perform a thorough comparison of the genetic variants identified from the FCA3DS study to those identified for Caucasian subjects in other studies

Mayo Clinic Biobank: samples and data on up to 1000 subjects from the Mayo Clinic Brain Bank. Most of these subjects do not have dementia and were selected from the clinic patients to undergo next-generation sequencing.

Alzheimer's Disease Sequencing Project: de-identified data from the ADSP cohort, which includes 10,000 subjects with whole exome, 5,000 subjects with whole genome, and an expected 5,000 subjects with follow-up sequence/genotype data.

UK10K: de-identified next-generation sequencing data from the UK10K cohort, which includes 10,000 subjects.

Collaborating institutions Mount Sinai Medical Center (Dr. Maria Greig-Custo) and University of Florida (Dr. Meredith Wicklund) had their IRB protocols approved (IRB#: 17-34-H-05 and IRB201700951, respectively).

Staff met with the study coordinators from both sites via phone conferences, and sent sample and data collection materials.

Staff completed an inventory of the African-American subjects in the database, and updated diagnoses and IRB information where applicable.

Staff continue to collect samples at Mayo Clinic Jacksonville and also continue receiving samples from Mount Sinai Medical Center. As part of the sample processing, demographic information on the collected samples are being entered into the database. To avoid any batch effects, staff plan to continue the collection and storage of the samples until the launching of whole exome sequencing (WES). Research staff plan to pursue WES in a single batch on the collected samples.

Researchers have collected a total of 61 African American participants (41 at mayo Clinic Florida ad 20 from Mount Sinai Medical Center) in year 1 (30 with AD or dementia, four with mild cognitive impairment=MCI, 27 cognitively intact controls). This is in addition to the existing cohort of 307 African American participants who do not currently have WES data (66 AD and 241 controls). Hence staff already have more than the required number of samples for which staff plan to perform WES (plan 63 AD and 62 control); therefore staff have accomplished the recruitment goal of Aim 1 and will launch WES in year 2.

Research staff took advantage of a new publication, which the research group also collaborated in (Sims, et al, Nature Genetics 2017), which identified novel AD risk variants in two genes (PLCG2, AB13) involved in innate immunity. To rapidly determine

whether these variants also pose risk in African Americans, staff have genotyped them in the existing African American cohort and are in the process of analyzing the data.

Aim 2:

Although staff had not planned on any studies for Aim 2, in year 1 of the project, staff also made progress on that front. In the original application staff had planned to perform transcriptome measurements in 250 PaxGene RNA samples and use the Nanostring nCounter platform for 30 genes. These 250 collections would come from the newly recruited patients, of which 125 will have WES. Although staff continue collect PaxGene RNA on the participants and currently have >70 PaxGene samples from the African American participants, there are some relative drawbacks to limiting transcriptome measurements to PaxGene RNA, including a lack of PaxGene RNA collections from most of the existing cohort, and availability of only a single time PaxGene collection due to cost. To overcome these drawbacks, staff optimized a novel method of measuring transcript levels in cell-free RNA from plasma. Researchers provide results from the optimization experiments below. Given these results, staff expect to be able to measure plasma cell-free transcript levels in 250 participants who will be prioritized for those that have WES data.

Researchers already have experience with successfully measuring transcriptome levels in the brain and PaxGene RNA using the Nanostring nCounter platform. Staff have now optimized plasma transcript measurements using the Nanostring nCounter Human Neuropathology panel for 760 gene transcripts. In the original proposal, staff had planned to measure expression levels of 30 AD candidate genes. Staff have since identified 50 candidate genes the transcript levels of which staff plan to measure using Nanostring nCounter platform. Importantly, staff already have the custom Nanostring chip designed. Hence, staff are poised to commence the planned transcript measurement studies in a higher number of transcripts than originally planned while staying within budget.

Aim 3:

As in Aim 2, although researchers had not planned on any studies for Aim 3 in the first year of the grant, staff have already made progress in that aim, as well. The goal in aim 3 is to utilize plasma Aβ and cognitive endophenotypes to discover and characterize AD risk genes and pathways in African Americans. As in Aim 2, staff will select plasma samples on 250 participants prioritized for having WES data. Also, although in the original submission staff had planned on obtaining plasma levels of Aβ42 only (Aβ40 and Aβ42), staff will be applying the novel Simoa HD-1 Analyzer to measure Aβ42, tau, and three cytokines (IL-6, IL10, TNFa, as part of the Cytokine 3-Plex kit), that will allow them to obtain levels of not only plasma Aβ, but also four other analytes from two different pathways (tau and innate immunity). Staff are currently in the process of sample selection for the studies in Aim 2 and Aim 3.

Follow On Funding: None at the time of reporting.

Collaborations: In this grant, the two collaborating institutions are University of Florida (Dr. Meredith Wicklund is site PI) and Mount Sinai Medical Center (Dr. Maria Greig-Custo is site PI). Staff have communicated with Dr. Todd Golde at the University of Florida for a collaboration of samples that they are collecting under their ADRC. Given that the patient population is predominantly Hispanic, this will provide an outstanding opportunity to determine role of AD risk variants in this minority population that is likewise under-represented in research. Staff have also launched a collaboration with Dr. Margaret Pericak-Vance at the University of Miami. Staff provided Dr. Pericak-Vance's team with the results of an ABCA7 deletion variant in the African American WES cohort. Dr. Pericak-Vance's team is in the process of genotyping some of the top genetic variants from the existing African American WES cohort in an independent African American series to seek replication. Further, staff have received approval from the Alzheimer's Disease Genetics Consortium to access their African American GWAS, which staff will utilize to seek additional replication for the top variants in the existing African American cohort.

Journals: Jun GR, Chung J, Mex J, Barber R, Beecham GW, Bennett DA, Buxbaum JD, Byrd GS, Carrasquillo MM, Crane PK, Cruchaga C, De Jager P, Ertekin-Taner N, Evans D, Fallin MD, Foroud TM, Friedland RP, Goate Am, Graff-Radford NR, Hendrie H, Hall KS, HamiltonNelson KL, Inzelberg R, Kamboh MI, Kauwe JSK, Kukull WA, Kunkle BW, Kuwano R, larson EB, Logue MW, manly JJ, Martin ER, Montine TJ, Mukherjee S, Naj A, Reiman EM, Reitz C, Sherva R, St George-Hyslop PH, Thornton T, Younkin SG, Vardarajan BN, Wang LS, Wendlund JR, Winslow AR, Alzheimer's Disease Genetics Consortium, Haines J, Mayeux R, Pericak-Vance MA, Schellenberg G, Lunetta KL, Farrer LA; *Transethnic genome-wide scan identifies novel Alzheimer's disease loci*; Alzheimer's Dement, July 2017.

Patents: None at the time of reporting.

11. **Grant #7AZ18:** Brain Amyloid Load and Novel Cognitive Measures in Diverse Ethnic Groups

    Principal Investigator: David Loewenstein, PhD

    Organization: University of Miami School of Medicine

    Progress Report: This research study on examining amyloid PET/CT scans in ethnically diverse populations has been very successful. The project results were reported in June 2018 at the Florida Alzheimer's Disease Summit and in Grand Rounds for Weil Cornell University, the University of Miami Miller School of Medicine, and Florida Atlantic University. Additionally, the study results were recently presented in Neurology Grand Rounds at the University of Miami Miller School of Medicine and slated to be presented in a special FDOH presentation in November of 2019.

    The study team reached the recruitment goal. The research team obtained 60 amyloid PET/CT scans from participants of diverse ethnicities (Hispanic and African Americans),

and the group of investigators is analyzing the data and preparing manuscripts regarding the study results for peer-reviewed publication.

As a result of critical pilot data obtained by this funding, researchers were awarded a five-year R01 grant from the National Institutes of Health that will facilitate this ongoing work. The pilot data generated by this study was also invaluable to a renewal application of the 1Florida Alzheimer's Disease Research Center, a state-wide consortium with the University of Florida, the University of Miami, and Mount Sinai Medical Center. Researchers anticipate funding of this center in 2020.

This investigation has significant impact for Floridians since it is the first to focus on the relationship between cognitive performance using a sensitive and new cognitive marker of preclinical AD, the failure to recover from proactive semantic interference, on a novel cognitive stress test (LASSI-L) and amyloid load and brain volumes. This grant created the infrastructure to do this work among diverse cultural groups that are an important growing segment of the US population (Hispanic and African-American individuals) at risk for Alzheimer's disease (AD). This is important in that the LASSI-L is sensitive to early biological changes in the brain related to AD, which can provide tools for early detection and treatment as more novel therapies become available. This focus on the relationship between these novel cognitive markers, amyloid load, and MRI findings represents a unique opportunity to contribute valuable knowledge to older adults which have been previously underserved in research.

Follow On Funding: National Institute on Aging - $2.6 million.

Collaborations: Florida International University - Malek Adouadi, PhD; CATE Center - Neuroimaging Processing of Amyloid PET Scans.

Journals: Rosie E. Curiel, Elizabeth A. Crocco, Ranjan Duara, Jessica M. Garcia, Monica Rosselli, Steven T. DeKosky, Glenn Smith, Russell Bauer, Cesar L. Chirinos, Malek Adjouadi, Warren Barker, David A. Loewenstein. A novel method of evaluating semantic intrusion errors to distinguish between amyloid positive and negative groups on the Alzheimer's disease continuum (Under Review). Biological Psychiatry.

Patents: None at the time of reporting.

12. **Grant #7AZ19:** Functionalized Intrabodies as Potential Anti-Tau Therapy

    Principal Investigator: Yona Levites, PhD

    Organization: University of Florida

    Progress Report: Alzheimer's disease (AD) is a complex neurodegenerative disorder with accumulation of amyloid beta and tau proteins. Many studies indicate that tau may be an attractive therapeutic target for the treatment of AD and other tauopathies. Immunotherapy is currently being pursued as a therapeutic approach to treating tauopathies and although there is data supporting that tau-targeted immunotherapies can be effective, it is unknown what constitutes an optimized tau immunotherapy. Efforts

to develop tau-targeted therapies have been hindered by a lack of *in vitro* assays which can predict *in vivo* efficacy, transgenic models which have slow and sometimes variable disease progression, and the limited ability of peripherally administered immunotherapies that reach the brain. In order to address these issues, research staff designed recombinant antibodies which can be targeted directly at the central nervous system using adeno-associated virus (AAV), and developed a novel pipeline in order to screen these potential therapeutics. Staff developed a novel method which allows for the rapid and cost-effective production of multiple rAAVs for use *in vitro* and *in vivo*. Staff then established that brain slice culture (BSC) paradigm predicts the *in vivo* efficacy of tau-targeted recombinant antibodies. Combining the BSC with an accelerated rAAV mouse model of tauopathy, staff identified specific recombinant antibodies targeting tau that are able to abrogate tau pathology and neurodegeneration both *ex vivo* and *in vivo*. Research staff also demonstrated that functionalizing these antibody fragments by fusing them to domains which alter their biologic properties may further improve their therapeutic potential. These studies constitute a doctoral thesis of a graduate student who is set to receive a PhD diploma in December 2019.

Follow On Funding: National Institute of Health - $419,375 (pending); National Institute of Health - $3,009,892 (not funded)

Collaborations: None at the time of reporting.

Journals: Marshall Goodwin, BS; Cara L. Croft; Hunter S. Futch; Daniel Ryu; Carolina Ceballos-Diaz; Xuefei Liu; Giavanna Paterno; Catalina Mejia; Doris Deng; Kimberly Menezes; Laura Londono; Kefren Arjona; Mary Parianos; Van Truong; Eva Rostonics; Amanda Hernandez; Sanford L. Boye; Shannon E. Boye; Yona Levites; Pedro E. Cruz; Todd E. Golde; *Utilizing minimally purified secreted rAAV for rapid and cost-effective manipulation of gene expression in the CNS*, Molecular Neurodegeneration, October 2019.

Patents: None at the time of reporting.

13. **Grant #7AZ20:** The Role of TTC3 in Alzheimer's Disease Pathogenesis

    Principal Investigator: Holly N. Cukier, PhD

    Organization: University of Miami

    Progress Report: Alzheimer's disease (AD) is the leading cause of dementia and currently affects about 500,000 Floridians. This proposal seeks to further investigate the relationship of AD with the ***tetratricopeptide repeat domain 3*** (*TTC3*) gene. The research group recently identified a potentially damaging mutation in the *TTC3* gene in a family of 11 individuals with AD (Cukier, et al, 2016). *TTC3* encodes an E3 ubiquitin ligase protein that plays an important role in AD pathways including Akt signaling, negative cell cycle control, and neuronal differentiation. Moreover, these pathways are intertwined with normal neuronal synaptic function, as well as Aβ and tau processing,

both features of AD pathology. This suggests that *TTC3* could play a protective role against AD; thus, mutations that reduce *TTC3* expression may contribute to AD risk.

The research group has generated three stem cell lines from AD individuals from this family that carry the mutation of interest in *TTC3*. Efforts are ongoing to define the characteristics of neurons generated from these cells as compared to neurons from control individuals to determine what the cellular features of these cells are that may contribute to AD. Research staff have also successfully used CRISPR genome editing to generate this *TTC3* mutation in a HEK cell line, which demonstrates that the tools that were designed are appropriate; however, efforts in the laboratory to repeat this genome editing in stem cells were unsuccessful. Staff outsourced this experiment to a company (Applied StemCell), that generated one homozygous cell line with the alteration. However, quality checks in the laboratory demonstrated that the isogenic control (original, unedited cell line) were mosaic for a genetic defect duplicating a portion of chromosome 20 (46,XY,dup(20(q11.2)). Therefore, the company is in the process of clonally isolating cells without this defect. Staff also have brains from members of this family who carry the *TTC3* mutation. Both brain tissue from these AD patients (old neurons in the late stages of disease) and growing neurons from stem cells of relatives with the *TTC3* mutation (young neurons in the very early stages of disease) will be used to assess expression profiles. Through additional data available from colleagues, this study will compare the brain and neuronal samples to samples of hundreds of unrelated AD individuals. Through the experiments outlined in this proposal, the research team aims to elucidate the function of *TTC3* and its potential role in cellular mechanisms that drive AD pathogenesis. Furthermore, by utilizing expression data available from additional AD individuals, this study could identify shared and unique pathways of disease and thereby provide a broader understanding of AD pathogenesis.

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 #7AZ23:** System Analysis of Potential Drug Interactions in the Treatment of Alzheimer's Disease from the FDA Reporting System, Electronic Health Records and Protein Interaction Networks

    Principal Investigator: Feng Cheng, PhD

    Organization: University of South Florida

    Progress Report: Two peer-reviewed papers have been published in journals. One additional paper is in preparation. Research staff gave a presentation at the International Conference on Intelligent Biology and Medicine (ICIBM 2018) and 128th Annual Florida Pharmacy Association (FPA) Meeting and Convention. Researchers

have enrolled and analyzed 378 participants in the USF Byrd Alzheimer's Center research study.

Follow On Funding: Alzheimer's Association Research Grant - $25,000

Collaborations: One PharmD student, Zahra Hasehmy, continued to work with Dr. Angela Hill to check the drug adverse events and drug prescription from the electronic health records of the Byrd Alzheimer's Institute.

Journals: Minh Pham, Feng Cheng, Kandethody Ramachandran; *A Comparison Study on Algorithms to Detect Drug-Adverse Event Associations: Frequentist, Bayesian*; Drug Safety, June 2018.

Patents: None at the time of reporting.

15. **Grant #7AZ24:** Correction of Tauopathy-Induced Circadian Dysfunction

Principal Investigator: Joshua Gamsby, PhD

Organization: University of South Florida

Progress Report: The research team's progress over the life of this grant has centered on further characterizing the circadian rhythm defect in a mouse model of tauopathy known as the Tg4510 mouse line, as well as trying to correct the defect. As part of the characterization of the circadian rhythm defect in the Tg4510 mouse line, staff initiated a longitudinal study that would determine when the circadian disruption occurs during the lifespan of the Tg4520 mouse line and better inform the treatment paradigm, which is planned for the second year of this award. The preliminary data presented in the original proposal demonstrated that eight-month old mice, which have advanced tauopathy and neurodegeneration, have a disrupted circadian clock; however, it was unclear if this disruption of circadian clock function happens relatively early during tauopathy progression in the Tg4510 mouse line – specifically, at three months of age. Again, this is very early in the pathology associated with the mouse line. For example, this is before neurodegeneration occurs. In 2017, research staff also demonstrated the circadian defect is evident at two months of age, which is quite surprising. These data suggest that the circadian disruption associated with the P301L tau mutation in the Tg4510 line is a very early event. Furthermore, staff tested another mouse line of tauopathy (the P19 transgenic line, which harbors the P301S mutation) for the circadian defect. The results show that the long circadian period defect is also evident in the PS19 line, but that this happens at a much later stage in the lifespan of the transgenic mouse. These data suggest that the specific type of tau mutation impacts the timing of when the circadian defect occurs. Additionally, staff showed that the long period defect also occurs in vitro, which complements the in vitro studies and provides staff with a suitable model of tauopathy-induced circadian disruption for future studies.

In the original proposal, staff endeavored to address tauopathy-induced circadian dysfunction in the Tg4510 line through the inhibition of GSK3β. GSK3β is an enzyme

that functions both to maintain normal timing of the molecular clock and to phosphorylate tau. Furthermore, GSK3β activity has been shown to be increased as a consequence of tauopathy, thus providing a potential mechanistic explanation for the disrupted circadian rhythm as normal GSK3β function is required to maintain normal timing of the molecular clock. As such, staff proposed to inhibit GSK3β in the Tg4510 line to rescue the tauopathy-induced circadian rhythm defects staff have observed. However, in light of recent findings from a collaborator at the Byrd Alzheimer's Institute (Dr. Danielle Gulick) demonstrating that inhibition of another kinase, casein kinase 1 (CK1), can rescue the period defect associated with a mouse model of beta-amyloidosis, staff chose to target this kinase instead. The first experiment was to inhibit both the delta and epsilon isoforms of CK1. Results from these show rescue of the long period defect in the Tg4510 line, suggesting that this kinase may be an attractive target to address the circadian defect associated with tauopathies.

Follow On Funding: NIH - $1,250,000 (not funded).

Collaborations: None at the time of reporting.

Journals: Stevanovic K, Yunus A, Joly-Amado A, Gordon M, Morgaan D, Gulick D, Gamsby J., *Disruption of normal circadian clock function in a mouse model of tauopathy*, Experimental Neurology, August 2017.

Patents: None at the time of reporting.

16. **Grant #7AZ25:** Understanding the Molecular Mechanisms of Seeding and Transmission of Wild Type and Mutant Tau

    Principal Investigator: Benoit Giasson, PhD

    Organization: University of Florida

    Progress Report: The accumulation of aberrantly aggregated tau defines a spectrum of tauopathies, including Alzheimer's disease, where the abundance and distribution of tau aggregates throughout the brain correlate with disease severity. Mutations in the tau gene (*MAPT*) cause frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17). Increasing evidence points to the importance of progressive tau self-inducing aggregation as a neurodegenerative mechanism. Initially using a high throughput cell culture model, research staff examined this property for an extensive spectrum of tau mutations causal of FTDP-17. Findings revealed stark differences between FTDP-17 tau mutants, with variants at amino acid proline 301 and serine 320 showing robust aggregation as compared to many other tau mutants. Similarly, staff elucidated the importance of certain tau protein regions and unique amino acid residues in the process. Overall, these differences alluded to potential mechanistic differences between wild type and some FTDP-17 tau variants in influencing tau aggregation. Furthermore, by combining two FTDP-17 tau mutants at proline 301 and serine 320, staff generated aggressive models of tauopathy that do not require exogenous induced seeding.

These findings were extended to generate novel and robust models of tau aggregation using viral-based transduction in mice and brain sliced cultures. Together, these studies provide novel insights in the molecular determinants that modulate tau aggregation. These models are already being used for the rapid screening of potential therapeutics to alleviate tau aggregation.

As such, researchers leveraged these findings and models to explore the role on serine 305 phosphorylation in tau aggregation. Staff demonstrated that molecular mimetic of serine 305 significantly inhibited tau aggregation. To further explore serine 305 phosphorylation in vivo, a monoclonal antibody (2G2) specific for tau phosphorylated at serine 305 was generated and characterized. Consistent with inhibition of tau aggregation, phosphorylation of serine 305 was not detected in pathological tau inclusions in Alzheimer's disease brain tissue. This study indicates that phosphorylation of unique tau residues can be inhibitory to aggregate formation and has important implications for potential kinase therapies. Additionally, it creates new tools for observing these changes in vivo.

Collectively, these studies provide novel insights in the specific molecular mechanisms influencing the induction and spread of tau pathology and the pathogenic consequences associated with tau aggregation and specific changes in tau protein.

Follow On Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Strang, K. H., Sorrentino, Z. A., Riffe, C. J., Gorion, K. M., Vijayaraghavan, N., Golde, T. E., Giasson, B.I. Phosphorylation of serine 305 in tau inhibits aggregation. Neurosci. Lett. 2018, 692:187-92. doi: 10.1016/j.neulet.2018.11.011. PubMed PMID: 30423399.

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Patents: None at the time of reporting.

17. **Grant #7AZ28:** Dementia Detection and Treatment Benefits Through Home Health Care as Compared to Clinic Care in a Rural Florida Underserved Population

    Principal Investigator: Lisa Kirk Wiese, PhD, MSN, RN, PHNA-BC, CNE

    Organization: Florida Atlantic University

    Progress Report: The purpose of this pilot grant funded by the Ed and Ethel Moore Alzheimer's Disease Research Foundation was to: 1) examine the influence of sociodemographic factors on Florida rural retiree willingness to participate in home-based cognitive screening, 2) determine effectiveness of home-based adult gerontological nurse practitioner (AGNP) diagnostic testing and referrals following screenings, and 3) evaluate the impact of this approach on Florida's ethnically diverse older residents facing a higher risk of Alzheimer's disease and related dementias (ADRD).

    The context of this study is that rural residents face twice the risk of developing ADRD and poorer outcomes as compared to urban residents for multiple reasons: increased comorbid disease, inadequate nutrition, low health literacy, internet access issues, and less access to specialty providers. However, early detection of dementia offers benefits such as discovering potentially reversible causes that can be treated, providing patients and families adequate time for planning future care, and initiating earlier treatment which may slow symptom progression. The Alzheimer's Association estimates that early and accurate diagnosis at the mild cognitive impairment stage in these higher risk rural settings could save up to 7.9 trillion dollars in health- and long-term care costs by 2050.

    The outcomes of this research showed that this approach of home-based screenings in rural subsidized housing units was highly successful: over half of the residents in three facilities participated in screening at just one offering. Of the 28 referred to the AGNP for further follow-up, 25 (89%) saw their provider. Of those, 16 (64%) began new therapy. As noted in the research proposal, early identification and treatment can lead to a *minimum* of one year of the client's ability to stay in their home - as compared to institutionalization. One year of aging-in-place results in an estimated cost savings of $33,000 per year per resident who is identified with dementia; in this study, this translates to an expected savings of $528,000 in just one year.

Implications for future work include the findings that ethnically diverse/underserved older adults' willingness to be screened for memory loss needs to be embraced by providers who have previously been hesitant to screen (due to inaccurate perceptions that persons do not want to know about an Alzheimer's disease diagnosis; see first publication). Other implications are that independent living facilities are an untapped source of research recruitment and intervention, and that engaging the day managers who are trusted by residents is essential. A third recommendation is that policy changes are needed that will support AGNPs to bill for dementia-focused geriatric assessments, even though the patient has a designated provider. The AGNP would then send the report to the provider for follow-up, as was successfully done in this pilot study. Indeed, providers offering feedback to the study unanimously reported welcoming the AGNP findings. They used the written report to begin conversations with their patients regarding possible prevention of further memory loss through treatment of chronic illnesses that can worsen dementia symptoms, and promotion of lifestyle changes such as increasing exercise and sleep patterns.

Follow On Funding: National Institutes of Health, National Institute of Aging - $379,242.

Collaborations: The Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL: Dr. James Galvin, Neurologist.

Journals: Wiese, L., Williams, C.L., & Galvin, J. (2018). Ethnically diverse rural Florida stakeholder perceptions about cognitive screening. Journal of *Aging and Mental Health*. https://doi.org/10.1080/13607863.2018.1525607

Wiese, L., Hain, D., Newman, D., Pangelley, C., Kaack, C., & Galvin, J, "Dementia among Older, Ethnically Diverse Residents of Rural Subsidized Housing" is under review for publication in the *Journal of Health Care for the Poor and Underserved.* Manuscript ID is JHCPU-Nov-2019-OP-0509.

Patents: None at the time of reporting.

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