Alzheimer's Disease Research Grant Advisory Board

Ed and Ethel Moore Alzheimer's Disease Research Program

2016-2017 Report

Rick Scott
Governor

Celeste Philip, MD, MPH
Surgeon General and Secretary of Health

2016-2017 Annual Report - Table of Contents

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

Alzheimer's disease is a debilitating brain disease that affects approximately 5.4 million Americans, including 510,000 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] 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 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, as required by section 381.82, Florida Statutes. Additional reporting requirements resulted through legislative change effective July 1, 2016 and are reflected in this report. This report provides additional information on the initial return on investment resulting from the state supported research grant funding.

ALZHEIMER'S DISEASE RESEARCH GRANT ADVISORY BOARD

OVERVIEW AND MEMBERSHIP

The Alzheimer's Disease Research Grant Advisory Board, authorized in section 381.82, Florida Statutes, advises the State Surgeon General on the scope of the research program. The Advisory Board may also provide advice on program priorities and emphases; oversight regarding mechanisms for the dissemination of research results; assistance in the development of appropriate linkages to nonacademic entities (such as volunteer organizations, health care delivery institutions, industry, government agencies, and public officials); in addition to any other responsibilities that may be requested. The Advisory Board consists of two gerontologists, two geriatric psychiatrists, two geriatricians, two neuroscientists, and three neurologists who may collectively submit recommendations for proposals to be funded to the State Surgeon General by the 15th of December each year. Grants shall be awarded by the State Surgeon General, after consultation with the Advisory Board, on the basis of scientific merit.

The names and positions of each Alzheimer's Disease Research Grant Advisory Board Member, as of December 10, 2016, are listed below (Biographical Statements or Curriculum Vitae is available upon request):

Gerontologists:
Leilani Doty, PhD, Chair
Florida ADRC (Alzheimer's Disease Research Center) Co-Principal Investigator, ORRE (Outreach, Recruitment, Retention, & Education) Core Leader
Past Director, University of Florida Alzheimer's Disease Initiative, Cognitive & Memory Disorder Clinics

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

Geriatric Psychiatrists:
Uma Suryadevara, MD
Assistant Professor, Department of Psychiatry, College of Medicine, University of Florida

Frederick Schaerf, MD, PhD
Director, Neuropsychiatric Research Center of Southwest Florida

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

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

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

Vacant position as of 12/2/16

Neurologists:
Ranjan Duara, MD
Medical Director, Wien Center for Alzheimer's Disease and Memory Disorders, Mount Sinai Medical Center

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

Vacant position as of 11/1/16

National Institutes of Health State Ranking
in Total Amount of Alzheimer's Disease Research Funding

Between fiscal years 2012-2014, Florida received an average of $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 nearly doubled to $22,729,691 in the state of Florida (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 seventh place and its total federal funding for Alzheimer's disease research increased by $10,518,757 in the 2015 fiscal year** (Figure 1). Florida is the only state in the southeastern United States to be ranked in the Top 10. This significant increase in federal research dollars may 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 3rd highest growth in new research funding, behind the states of Georgia and Ohio, and saw the highest funding gains of the Top 10 ranked states in 2015, nearly double the rate of the 2nd ranked state, Massachusetts** (Figure 2 and 3).

National Institutes of Health Alzheimer's Disease Research
State Funding and Rankings Fiscal Year 2015

State	Total Funding	Rank
CA	$94,496,399	1
MA	$65,518,366	2
NY	$64,474,845	3
TX	$47,711,416	4
PA	$32,190,938	5
WA	$24,063,891	6
FL	**$22,729,691**	**7**
MD	$22,405,435	8
IL	$19,746,232	9
MO	$19,200,195	10
NC	$17,610,947	11
MN	$17,015,821	12
GA	$12,484,961	13
OH	$11,473,759	14
KY	$8,798,087	15
TN	$7,403,871	16
MI	$6,910,262	17
AZ	$6,869,424	18
IN	$5,857,991	19
CT	$5,730,026	20

Figure 1 NIH Research Funding from the 2015 Fiscal Year Reporting Period: The top twenty ranked states in NIH funding for Alzheimer's disease is displayed. With over $22.7 million in NIH funding, Florida is ranked 7th in the nation.
Source: National Center for Health Statistics, National Institutes of Health 2016

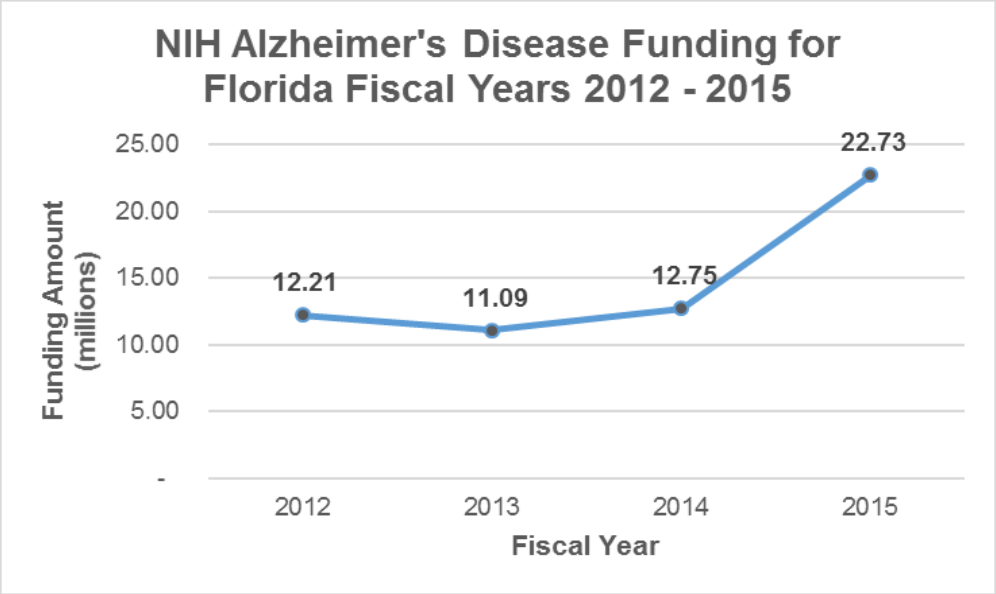

Figure 2 NIH Research Funding Trends in Florida Fiscal Year 2012-2015: This chart illustrates the recent trends in federal funding for Alzheimer's disease research in the state of Florida. Following three years of relative stability in funding levels, the 2015 fiscal year saw a sharp increase to $22.7 million, nearly double the amount in 2014. *Source: National Center for Health Statistics, National Institutes of Health 2016*

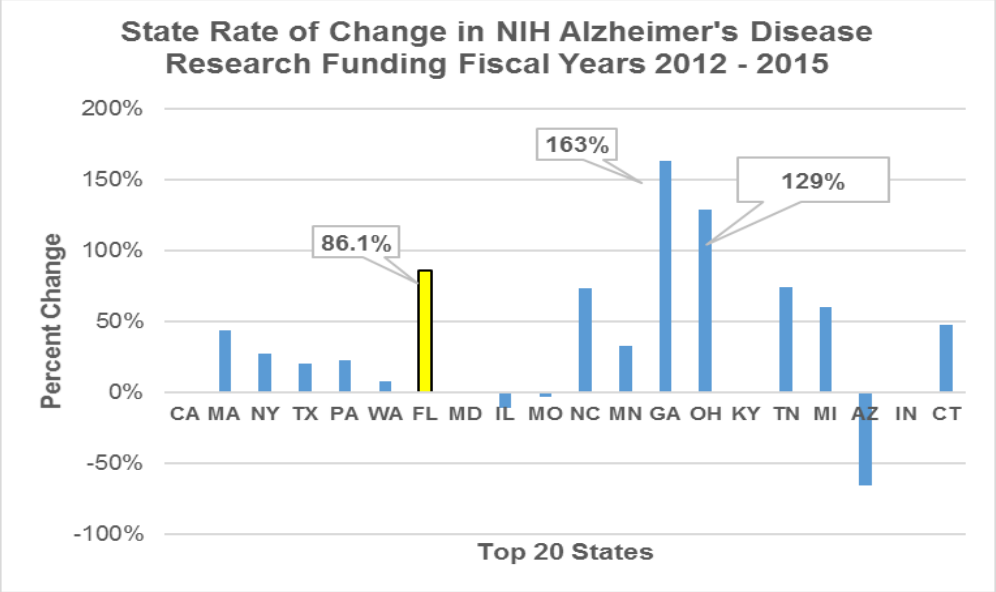

Figure 3 Change in NIH Research Funding in the Top 20 States Fiscal Years 2012-2015: This graph displays the rate of change in federal Alzheimer's disease research funding for the Top 20 States for fiscal years 2012-2015. Amongst the Top 10 ranked states in NIH funding, Florida saw the highest funding gains in 2015. The rate of change is almost double the rate of Massachusetts, the 2nd fastest growing rate in the Top 10 and the 2nd ranked state of NIH funding for Alzheimer's disease. At 86.1%, Florida saw the third-highest growth, behind Georgia and Ohio. Most states saw increased levels of funding, except Illinois, Missouri, and Arizona, which saw decreased funding. California, Maryland, Kentucky, and Indiana saw little to no change, overall. *Source: National Center for Health Statistics, National Institutes of Health 2016*

PROGRESS TOWARD PROGRAMMATIC GOALS

The Ed and Ethel Moore Alzheimer's Disease Research Program 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 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 increased funding from $3 million to $5 million. An additional $36,000 of unencumbered funds were added to the $5 million allocation for grant awards. This increased funding resulted in awarding 27 research grants. Appendix A details all newly awarded grants. Additionally, Appendix B details all active grants, and Appendix C details all closed grants in 2016-2017. Information regarding progress reports, follow on funding, publications and patents of each active and closed grant is also found in Appendix B and C, respectively.

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 Research Grant Program with increased allocated funding to award $5,036,000 to 27 outstanding researchers in Florida. Without this support, the eminent scientific advancements and discoveries in Alzheimer's disease would not be possible. Although great bounds have occurred, the Advisory Board requests that 10% of the funds allocated to this program be made available for administrative expenses, which is consistent with other research programs administered by the Department. These funds will be used to recruit peer-reviewers, especially in basic research science, to assist the Advisory Board in their evaluation of grant applications. In addition, statutory change is needed to reimburse travel expenses resulting from Advisory Board in-person meetings. In-person meetings increase strategic planning and in-depth communication about critical research issues. Implementing these recommendations will assist in elevating this research program to new heights.

The Alzheimer's 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 2016-2017 NEWLY AWARDED GRANTS (effective December 8, 2016)

Grant #	Organization	PI Name	Project Title	Award Amount	End Date
7AZ01	University of Florida	Smith, Glenn	Consortium Study of Neuroimaging Impact of Behavioral Interventions in Mild Cognitive Impairment	$ 450,000	3/31/2021
7AZ02	Florida International University	Burke, Shanna	Demographic, Neuropsychological and Functional Classification, Risk Factors, and Progression Rates of Individuals in the National Alzheimer's Coordinating Center Database using Algorithmic Diagnosis.	$ 99,994	3/31/2019
7AZ03	University of South Florida	Kang, David	Structure Activity Characterization of Novel Slingshot Inhibitors	$ 250,000	3/31/2021
7AZ04	University of Miami	Rotundo, Richard	Enhanced Acetylcholinesterase Expression Induced by Donepezil and Galantamine	$ 250,000	3/31/2021
7AZ05	Mayo Clinic Florida	McLean, Pamela	How does alpha-synuclein contribute to tau dysfunction in AD?	$ 250,000	3/31/2021
7AZ06	University of Florida	Bizon, Jennifer	Impact of perirhinal cortical tau pathology on pre-clinical cognitive decline	$ 100,000	3/31/2019
7AZ07	Mayo Clinic Florida	Carrasquillo, Minerva	Early detection biomarkers of Alzheimer's disease inflammation and vascular risk factors in African Americans	$ 250,000	3/31/2021
7AZ08	Mayo Clinic Florida	Dickson, Dennis	Pathophysiology of Traumatic Brain Injury in the State of Florida Alzheimer's Disease Initiative Brain Bank	$ 100,000	3/31/2019
7AZ09	University of Miami	Harvey, Philip	Post-doctoral Research Fellowship	$ 86,792	3/31/2019
7AZ10	University of Florida	Janus, Christopher	Corticotropin-releasing hormone (CRH) Immunotherapy for Alzheimer's disease	$ 100,000	3/31/2019
7AZ11	University of Central Florida	Sugaya, Kiminobu	Antibody targeting of IL1RAP and studying their therapeutic effects in mouse models of Alzheimer's disease	$ 100,000	3/31/2021
7AZ12	University of Florida	Rincon-Limas, Diego	Large-scale identification of genes that suppress concurrent Abeta42 and tau pathology in vivo	$ 249,998	3/31/2021
7AZ13	University of South Florida	Gulick, Danielle	CK1 delta inhibition to reduce sundowning in Alzheimer's disease	$ 100,000	3/31/2019
7AZ14	University of Miami	Curiel, Rosie	A Consortium to Study Precision-based Computerized Assessment for the Detection of Mild Cognitive Impairment in Older Adults	$ 249,846	3/31/2021
7AZ15	Mayo Clinic Florida	Allen, Mariet	Identification of functional regulatory variants at Alzheimer's disease loci	$ 100,000	3/31/2019
7AZ16	Mayo Clinic Florida	Heinzelman, Pete	Yeast Surface Display Engineering of Human Fibronectin Domains for Enhanced Brain Delivery of Alzheimer's Disease Therapeutics	$ 95,133	3/31/2019
7AZ17	Mayo Clinic Florida	Ertekin-Taner, Nilufer	Florida Consortium for African-American Alzheimer's Disease Studies (FCA3DS)	$ 308,807	3/31/2021
7AZ18	University of Miami	Loewenstein, David	Brain Amyloid Load And Novel Cognitive Measures in Diverse Ethinic Groups	$ 249,980	3/31/2021
7AZ19	University of Florida	Levites, Yona	Functionalized Intrabodies As Potential anti-Tau Therapy	$ 250,000	3/31/2019
7AZ20	University of Miami	Cukier, Holly	The Role of TTC3 in Alzheimer's Disease Pathogenesis	$ 250,000	3/31/2021
7AZ21	Mayo Clinic Florida	Cook, Casey	Evaluating the mechanism by which TauA152T modulates risk of tauopathy	$ 250,000	3/31/2021

7AZ22	Mayo Clinic Florida	Kanekiyo, Takahisa	APOE and cerebrovascular aging in Alzheimer's disease	$ 250,000	3/31/2021
7AZ23	University of South Florida	Cheng, Feng	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	$ 98,449	3/31/2019
7AZ24	University of South Florida	Gamsby, Joshua	Correction of Tauopathy-induced Circadian Dysfunction	$ 100,000	3/31/2019
7AZ25	University of Florida	Giasson, Benoit	Understanding the molecular mechanisms of seeding and transmission of wild type and mutant tau	$ 250,000	3/31/2021
7AZ26	University of Miami	Wahlestedt, Claes	Preclinical investigation of an optimized formulation of resveratrol, JOTROL, for Alzheimer's disease	$ 100,000	3/31/2021
7AZ27	University of Central Florida	Tatulian, Suren	Structure and Toxicity of Amyloid Beta Hetero-Oligomers	$ 100,000	3/31/2021

NEW GRANTS FISCAL YEAR 2016-2017

(Funding Year 2016-2017)

1. **Grant #7AZ01:** Consortium Study of Neuroimaging Impact of Behavioral Interventions in Mild Cognitive Impairment

   Principal Investigator: Glenn E. Smith, PhD

   Organization: University of Florida

   Abstract of Proposed Research: There are currently no effective medications for those with Mild Cognitive Impairment (MCI) due to Alzheimer's or other diseases. Rather, behavioral interventions, especially cognitive remediation interventions, provide the most useful approach to addressing the behavioral and social needs of those with MCIs. The Principal Investigator (Dr. Smith) and Co-Principal Investigator (Dr. Chandler) for this proposal have a long-standing research collaboration examining which behavioral interventions may be most helpful in delaying progression to dementia for people with MCI. In this project, a three-site consortium will be established to extend this research and add to the number of Florida Memory Disorders Clinics that have the capacity to do this kind of behavioral research and offer this kind of clinical service. In addition to the central aim of expanding clinical research capacity in Florida this grant is configured to address a critical scientific question. It will compare two promising behavioral interventions (computerized brain fitness and yoga) to each other and to a control arm (wellness education). The impact on cognition, function and quality of life will also be studied. Moreover, neuroimaging will be used to estimate the post-intervention neuronal plasticity changes associated with this behavioral intervention in people with Mild Cognitive Impairment. The long-range goal is to build a network of Memory Disorders Centers with the capacity to test hypotheses that both behavioral interventions, including brain fitness and mind-body (yoga) cognitive remediation strategies will aid in slowing the progression of mild cognitive impairment through different mechanisms. Brain fitness programs will primarily improve cognitive function by increasing the functional integrity of the brain's cortical hubs (highly connected regions) due to more efficient information

processing, while yoga primarily will increase global and regional cerebral perfusion. If these effects are present we will determine if either or both mechanisms also decrease pathology-related atrophy. This effort will enlarge this collaborative team, and generate a robust Florida network for behavioral intervention research and delivery of 'prevention' services. This proposal will also provide preliminary data for a subsequent longer, larger, Florida-led, multisite study to be submitted to the National Institute of Aging.

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

   Abstract of Proposed Research: This study seeks to trial an enhancement of the diagnostic algorithm that intends to classify individuals based on results from neuropsychological testing and clinical dementia ratings. Specifically, this study plans to objectively test the central hypothesis by pursuing the following goals. First, older adults will be self-classified into well-defined cognitive status entities, such as cognitively normal, impaired but not Mild Cognitive Impairment (MCI), amnestic MCI, non-amnestic MCI, and dementia, using a diagnostic algorithm that considers a combination of amnestic and non-amnestic neuropsychological tests scores and its assigned clinical dementia rating. We will, then, explore the participants' demographic characteristics, protective factors, and risk factors associated with cognitive status subtypes. Once the cognitive subtypes are established, as well as, their associated characteristics, risk factors, and protective factors, the rates of progression from one cognitive subtype to another, over a period, will be examined. This expert system, algorithmic diagnostic software, will be freely distributed as open source software and available for download on the popular open source software sharing site, github.com. The proposed study is directly applicable to Priority Area 3, with a specific focus on area 3.5: expert diagnosis system. In under resourced and understaffed health care settings, the technology proposed herein has the potential to allow free-standing memory disorder clinics and primary care facilities to provide the expert detection and diagnostic services generally delivered by University Centers. Given that the Alzheimer's disease pathophysiological process likely begins 10 to 20 years prior to any observable symptoms, it is crucial to understand the early contributing risk factors, which may be revealed through an algorithm that can quickly, precisely, and simultaneously account for multiple variables. According to the Pew Research Institute (2015), 53 of 67 counties in Florida have an above-average share of people 65 and older when compared with the percentage (14.5%) of Americans (46.2 million) in that age range. This population is expected to grow over the next 20 years. The Department of Elder Affairs in the State of Florida reports "there are 500,000 individuals living with Alzheimer's disease [in Florida]. By 2020, it is anticipated that 580,000 individuals will be living with Alzheimer's disease. Nearly 12% of Florida senior population has been diagnosed with Alzheimer's disease." As the state's population grows, yet economic resources remain stagnant, it is imperative to focus on cost-saving strategies to provide memory care diagnosis and services to a growing elderly population. This project has the potential to affect not only a large share of the current aging population in Florida, but, also, the country, as Florida

continues to attract older adults in the winter months and as a prime retirement destination.

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

   Principal Investigator: David Kang, PhD

   Organization: University of South Florida

   Abstract of Proposed Research: Alzheimer's disease (AD) is a devastating neurodegenerative disorder of the brain that afflicts more than 5.4 million people in the United States and close to 500,000 people in Florida. At present, however, there are no effective treatment or therapy for AD. Two major pathologies – namely amyloid plaques and tau tangles – are responsible for the neurodegenerative changes seen in AD brains. While amyloid pathology is thought to initiate AD, tau is essential to execute the progressive neurodegeneration seen in AD. Previous studies in this lab have found that the Slingshot-Cofilin pathway not only promotes amyloid production but also links amyloid with tau pathologies. Recently, this lab has identified several promising Slingshot inhibitor compounds that not only reduce amyloid production but also inhibit the toxic amyloid signaling to tau. In this project, a combination of chemical, biochemical, cellular, structural, and computational techniques will be used to determine the structure-activity-relationship between the compounds and Slingshot activity, focused on pathologically-relevant outcomes. This will allow for the optimization of these small molecule compounds to more effectively target the pathological process. As such, the results of this study are expected to lead to the identification of promising novel drug-like compounds that can potentially combat AD, as well as, determine the mechanisms of action of Slingshot and Cofilin in modifying AD pathology.

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

   Principal Investigator: Richard L. Rotundo, PhD

   Organization: University of Miami

   Abstract of Proposed Research: Acetylcholinesterase (AChE) is the enzyme responsible for terminating neurotransmission at cholinergic synapses in the central and peripheral nervous systems in virtually every animal species. For this reason, tens of thousands of AChE inhibitors have been developed over the past 80 years for use as pesticides, nerve agents and therapeutic drugs for the treatment of disorders such as myasthenia gravis and Alzheimer's disease. The underlying assumption in all these applications is that AChE inhibitors act solely to reduce or eliminate its catalytic activity thereby increasing available acetylcholine at the synapse. In contrast, unpublished preliminary studies in our lab show that a subset of these inhibitors, such as those used for the treatment of dementias, also act as pharmacological chaperones to enhance the

folding of newly-synthesized AChE. This in turn increases the production of catalytically active enzyme molecules. The net result is an increase in the synaptic form of AChE in the CNS with the potential to reverse the desired effects of these drugs. In addition, these results suggest a plausible explanation for the "sundown" effect observed in many Alzheimer's patients where their symptoms appear worse at the end of the day after taking these drugs. The specific aims of this proposal are: 1) to determine in detail using tissue cultured cells which types of AChE inhibitors enhance enzyme folding as opposed to only inhibiting enzyme activity, the desired effect for the treatment of Alzheimer's disease; 2) to determine whether they exert the same effects on AChE folding in vivo compared to carbamate type inhibitors such as rivastigmine or neostigmine using a mouse model; 3) to test the hypothesis that a combination of an active site directed inhibitor such as donepezil or galantamine together with a carbamate type AChE inhibitor such as rivastigmine or neostigmine, anticholinesterases already in clinical use that are predicted to not enhance AChE folding, may give superior memory retention using a mouse model. These studies will clarify the molecular mechanisms of this novel and unpredicted side effect of the two major drugs used for treating Alzheimer's disease. More importantly, they will provide a possible solution to the problem by reducing the effects of these drugs on AChE folding while maintaining elevated acetylcholine levels through sustained inhibition using alternative AChE inhibitors.

5. **Grant #7AZ05:** How Does Alpha-Synuclein Contribute To Tau Dysfunction In AD?

   Principal Investigator: Pamela McLean, PhD

   Organization: Mayo Clinic Jacksonville

   Abstract of Proposed Research: The main pathological features of Alzheimer’s disease (AD) are the formation of plaques and neurofibrillary tangles in the brain, composed of beta-amyloid (Abeta) and MAPT (tau) proteins, respectively. In another form of dementia called dementia with Lewy bodies (DLB), as well as, Parkinson’s disease (PD), alpha-synuclein (asyn) is the major pathological protein. Although the aggregation of Abeta, tau, and asyn are used as the major pathological markers of AD and PD, respectively, there is ample evidence that these pathogenic proteins are closely linked in neurodegenerative diseases. Importantly, AD patients with asyn pathology usually present with a more rapid cognitive decline and shortened survival time compared to AD patients without asyn pathology. In human Alzheimer’s disease brains, tau and asyn pathology are often found together in the same neuron. There is also increasing evidence that tau is a presynaptic protein, much like asyn, and that tau and asyn may interact at cellular membranes. In this application, this lab will try to determine if there are previously undetected forms of asyn and tau in Alzheimer disease postmortem brains that could contribute to disease, and we will use neurodegenerative model systems to probe a role for tau-asyn interactions in the progression of Alzheimer’s disease and other dementias. Human post-mortem brain samples, from the Mayo Clinic Brain Bank, will be used to determine if a tau-asyn interaction is prevalent in AD

compared to other neurodegenerative diseases and healthy controls. In addition, a novel mouse model with abundant tau pathology and the associated behavioral phenotype will be used to determine if co-expression of asyn exacerbates the phenotype, shortens survival time, and increases pathology. This project addresses the objectives of the Ed and Ethel Moore Alzheimer's Research Program by proposing to validate asyn as a novel therapeutic target for AD and by providing insight into possible pathological mechanisms. Investigating asyn as a target for therapeutics is appropriate, given the considerable evidence that AD is a complex proteinopathy, which commonly has comorbid asyn pathology, and displays overlapping symptoms with other neurodegenerative diseases

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

   Principal Investigator: Jennifer Bizon, PhD

   Organization: University of Florida

   Abstract of Proposed Research: Accumulation of tau proteins is a pathological hallmark of Alzheimer's disease that initially emerges in a brain region referred to as the transentorhinal subregion of the perirhinal cortex. Viral-based technology, which allows human pathological genes to be expressed in animal models, has been useful for conducting preclinical investigation to better understand how tau proteins contribute to the development and progression of Alzheimer's disease. These preclinical models, however, have, thus far, exclusively employed young subjects and have not yet incorporated the neuroanatomical features of human disease pathology. Even in the absence of pathology, the aged brain has several biological features that differ from young subjects and that could influence disease processes. As Alzheimer's disease develops against the backdrop of an aging brain, it is critical to elucidate how aging and pathological tau interact to influence disease mechanisms and cognitive outcomes associated with Alzheimer's disease. The first goal of this research is to establish a rat model of pre-clinical Alzheimer's disease in which viral-mediated gene transfer will be used to drive the expression of human toxic tau species in the perirhinal cortex of an aged rat. The perirhinal cortex receives input from all sensory modalities, and is critical for the perception of highly processed sensory representations integral for memory formation. This lab has developed and validated highly sensitive behavioral assays of perirhinal cortical function that strongly predict memory in preclinical animal models. The secondary goal of this proposal is to establish these behavioral assays as a biomarker for early detection and tracking of disease pathology in patient populations.

7. **Grant #7AZ07:** Early Detection Biomarkers Of Alzheimer's Disease Inflammation And Vascular Risk Factors In African Americans

   Principal Investigator: Minerva M. Carrasquillo, PhD

Organization: Mayo Clinic Jacksonville

Abstract of Proposed Research: Alzheimer's disease (AD) is a growing epidemic that is having an increased impact on society as life expectancies rise. Up to 74% of the risk for AD can be attributed to genetic factors; therefore, improving our knowledge of the underlying genetic risk factors is essential to our understanding of the disease pathomechanism, and for the development of treatments and prevention. Although AD is twice as prevalent in African-Americans as in subjects of European descent, the vast majority of genetic studies aimed to identify AD risk factors have been limited to Caucasian populations. Given that there is also a higher risk of cardiovascular disease in African-Americans, and the strong evidence for a link between vascular disease and AD, the long-term goal of this proposal is to improve the understanding of the influence of vascular disease risk factors and inflammation on AD in this minority population. Specifically, this proposal aims to identify genetic variants that influence genes involved in inflammation or vascular function, in African-Americans, and to develop minimally invasive blood and plasma biomarkers to aid in early disease diagnosis. The knowledge gained from this study could also lead to new and more relevant treatments, and ultimately preventive therapies. This funding opportunity will enable targeted analysis of genes/proteins involved in inflammation and vascular health through (1) a thorough screen for genetic variants that associate with AD in the Mayo Clinic African-American AD case-control series in targeted genes, (2) identification of altered blood gene expression or plasma protein levels, in AD vs. non-ADs for targeted genes/proteins (3) development of early detection biomarkers through the correlation of AD age-at-onset with (a) blood gene expression and (b) plasma protein levels of targeted genes. The current application would address all three focus areas in Priority Area 4 of the 2016 Funding Opportunity Announcement released by the Florida Health, Ed and Ethel Moore Alzheimer's Disease Research Program, as the proposed studies focus on AD in African-Americans, an understudied population in which AD is twice as prevalent as in Caucasians (Focus Area 4.1.), aim to identify vascular risk factors that contribute to AD (Focus Area 4.2), and aim to develop minimally invasive early detection biomarkers (Focus Area 4.3).

8. **Grant #7AZ08:** Pathophysiology Of Traumatic Brain Injury In The State Of Florida Alzheimer's Disease Initiative Brain Bank

   Principal Investigator: Dennis W. Dickson, MD

   Organization: Mayo Clinic Jacksonville

   Abstract of Proposed Research: Traumatic brain injury (TBI) is a strong environmental risk factor for the development of dementia, including Alzheimer's disease (AD). The associative risk between TBI and dementia has been reported to be 'dose-dependent', or based on the severity of TBI and number of TBI. In this regard, repetitive TBI can result in a neurodegenerative disorder known as chronic traumatic encephalopathy (CTE). The most well-defined sources of repetitive TBI that can lead to CTE are sustained through contact sports participation (football, boxing, soccer, wrestling, and

others) or military blast exposure (improvised explosive devices). CTE is a neuropathologically-defined disorder with characteristic abnormal deposits of the protein tau in neurons and astrocytes at the depths of folds in the brain ('cerebral sulci') and surrounding blood vessels. While CTE pathology may exist as the sole brain pathology in certain cases, many cases (especially older individuals) harbor comorbid brain pathologies consisting of CTE, as well as, other neurodegenerative pathologies. Senile plaques, the hallmark lesions of AD, are observed in over half of CTE cases, and have been reported to increase with CTE severity. Due to the complex relationship between TBI, CTE, and AD, there exists a need to clarify 1) how TBI can lead to these combined pathologies, 2) whether the presence of CTE pathology modifies AD pathology and vice versa, 3) how the combination of CTE and AD affects the clinical picture of dementia, and 4) whether there are specific risk factors which predispose individuals to both CTE and AD. In this proposed study, we will search for CTE and other TBI pathologies in the Alzheimer's Disease Initiative (ADI) Brain Bank, a brain banking program sponsored by the state of Florida's Department of Elder Affairs. Within the ADI Brain Bank, 1,004 brains meet neuropathology diagnostic criteria for AD. It is proposed to screen these brains for CTE tau pathology and comparing these findings to information extracted from clinical records pertaining to demographics (gender, race, education, alcohol/tobacco use), neurodegenerative disease (family history, disease onset, disease duration, age at death), traumatic brain injury (sporting-related trauma, non-sporting related trauma), psychiatric impairment (depression, anxiety, obsessive compulsive disorder, post-traumatic stress disorder), and clinical cognitive assessment scores. Finally, using DNA (deoxyribonucleic acid) from these 889/1,004 cases, we will attempt to identify genetic risk factors in cases with CTE and TBI pathology not found in cases without CTE and TBI pathology. These findings will give important insight toward understanding the pathophysiology of TBI and its contribution to AD progression.

9. **Grant #7AZ09:** Post-doctoral Research Fellowship

   Principal Investigator: Philip Harvey, PhD

   Organization: University of Miami

   Abstract of Proposed Research: There is a pressing need to train promising researchers to study more innovative ways of assessing and diagnosing persons in the earliest stages of Alzheimer's disease (AD). Early diagnosis paves the way for increasingly more targeted treatment interventions. This application presents an unprecedented opportunity for post-doctoral neuropsychology fellowship training to a) study the earliest Preclinical manifestations of AD; b) participate in the development and implementation of novel measures to assess Pre-clinical AD; c) learn to clinically evaluate different ethnic and cultural groups for early stage mild-cognitive impairment; d) learn how to interpret and to conduct research relating cognitive and functional test findings to biological measures of the brain and e) learn to publish papers and prepare NIH funded applications for further extramural grant support. The primary mentor for the fellow would be Philip Harvey, PhD, a prominent neuropsychologist and scientist who has a specialty in cognition, aging, and the development of novel functional assessment

tools. Dr. David Loewenstein, PhD, ABPP, a board-certified neuropsychologist, Director of the Division of Neuropsychology and Professor of Psychiatry and Behavioral Sciences at the Miller School of Medicine at the University of Miami Miller School of Medicine would be the fellow's Primary Co-Mentor. Together, Drs. Loewenstein and Harvey have pioneered novel functional assessment in neurologically vulnerable individuals including those at risk for AD. Dr. Loewenstein is currently the Principal Investigator (PI) of a five year National Institutes of Health (NIH) R01 grant studying novel cognitive paradigms for the prediction of cognitive decline in the elderly. He is also co-leader of the Clinical Core and Scientific Project Director of the newly funded Alzheimer's Disease Research Center (ADRC) located at Mount Sinai Medical Center that relates novel cognitive and brain biomarkers to cognitive decline in Preclinical AD in Hispanic and Non-Hispanic populations. Dr. Harvey would also be assisted by co-mentor Dr. Rosie E. Curiel, (University of Miami); an Assistant Professor and co-investigator on the abovementioned projects who actively mentors an Ed and Ethel Moore postdoctoral fellow. Dr. Curiel is a geriatric neuropsychologist who focuses on cross-cultural neuropsychological assessment. These mentors can provide an unprecedented opportunity for a post-doctoral fellow to gain extensive research and clinical diagnostic experience. The proposed fellowship will help cultivate fresh talent into the critical area of early diagnosis of older adults representing different cross-cultural groups, provide excellent academic mentorship by distinguished investigators and clinicians and prepare the individual for a successful career in clinical patient oriented research.

10. **Grant #7AZ10:** Corticotropin-Releasing Hormone (CRH) Immunotherapy For Alzheimer's Disease

    Principal Investigator: Christopher Janus, PhD

    Organization: University of Florida

    Abstract of Proposed Research: Alzheimer's disease (AD) is the most widespread cause of dementia among elderly populations, affecting more than 37 million people worldwide according to the 2009 census. Recent clinical reports indicate that chronic stress may significantly increase the risk of developing AD. Also, other stress related diseases, like posttraumatic stress disorder or depression, significantly increase risks for the development of dementia. The physiological response to stress is the activation of hormonal response in the brain and adrenal glands (so called hypothalamic-pituitary-adrenal axis (HPA)), with the purpose to restore the hormonal balance of the body. The small peptide, called corticotropin-releasing hormone (CRH) constitutes the primary response to stress. If stress persists, then the excessively higher levels of CRH lead to long-term dysregulation of HPA, which causes increases in levels of amyloid beta (Aβ) and tau abnormal phosphorylation, as well as abnormal behavior of AD patients. The consequent chronic increased levels of plasma cortisol correlate with neuronal death in the brain and cognitive deficits, leading to AD dementia. It is proposed to selectively lower the levels of CRH in the brain with the purpose to stave off the cascade of deleterious pathological events leading to AD dementia. To this end, using mouse models of behavioral stress, it is proposed to test novel immunotherapeutic approaches to decrease CRH signaling in the brain. Initial data showing this lab's ability to induce a

robust antiCRH response with a synthetic vaccine has already been collected. In this pilot study, the aim is to identify an optimized vaccination approach and to generate proof of concept data that will substantiate the hypothesis that lowering CRH levels prevents cognitive decline in a stressed mouse. These results will provide compelling evidence that CRH might be a viable potential target for intervention in AD.

11. **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

    Abstract of Proposed Research: Alzheimer's disease (AD) is a devastating disease caused by a breakdown of brain networks involved in memory function. The disease pathology is multi-dimensional and several pathways are involved in disease progression. In AD, amyloid-β peptide (Aβ) is one the main proteins involved in pathology of AD. There is currently no therapy proven to stop or reverse the underlying cause of the progressive symptoms of Alzheimer's disease. Research needs to be focused on newer gene mechanisms that are responsible for the clearance of amyloid plaques, particularly in early stages of the disease (when symptoms are mild or not yet present). Recently, researchers have reported a variant in a gene (IL1RAP) associated with greater amyloid plaque accumulation. Based on several studies, it is suggested that targeting the protein (IL1RAP) will be a viable approach for faster clearance of amyloid deposits and for improvement in controlling Alzheimer's disease. It is proposed to use exosomes as delivery vehicles to deliver antibody that can stop the activity of IL1RAP, in order to decrease amyloid-β peptide formation in the Alzheimer mouse model. Brain cell (oligodendroglial) exosomes will be used as delivery vehicles. Recent studies have shown these exosomes are involved in improving the brain integrity. The important aspect is to increase the specificity target delivery of these exosomes. The surface of exosomes will be engineered to display brain homing peptides (BHP1). The BHP1 peptide will specifically direct the exosomes to the brain cells. The therapeutic antibody against IL1RAP will be attached to the exosomes using click chemistry. Alzheimer's disease patient derived induced pluripotent stem cells (iPS cells) will be used to study the effect of these new therapeutic delivery systems. Alzheimer's disease mouse models will also be used to study this novel therapeutic approach utilizing exosomes delivering antibodies to arrest the IL1RAP activity. The functional outcomes by the antibody treatment will be determined using magnetic resonance imaging (MRI), and behavioral and histological analysis. A transgenic mouse model or appropriate mouse model will be utilized to represent the Alzheimer disease pathology to study the effects of exosomal delivery of antibodies against IL1RAP. Exosomes have huge potential in utilization as a delivery vehicle; however, few studies have been done to utilize exosomes as antibody delivery vehicles in Alzheimer's disease. Exosomes have a natural ability to internalize into the cells and can exploit this nature of exosomes to deliver therapeutic proteins to neuronal cells.

12. **Grant #7AZ12:** Large-Scale Identification Of Genes That Suppress Concurrent Abeta42 And Tau Pathology In Vivo

Principal Investigator: Diego E. Rincon-Limas, PhD

Organization: University of Florida

Abstract of Proposed Research: Alzheimer's disease (AD) is an incurable neurodegenerative disorder that causes progressive memory loss and cognitive impairment, leaving patients totally incapacitated. The two landmark lesions in Alzheimer's disease (AD) are extracellular amyloid plaques mainly formed by the amyloid beta-42 (Abeta42) peptide and intracellular neurofibrillary tangles containing aggregates of abnormal tau protein. Abeta42 and tau were thought of as independent culprits for a long time, but in light of recent studies, it is clear that they are intimately related and have synergistic activities. However, very little is known about how (and which) Abeta and tau interactions trigger AD pathogenesis, which significantly impedes the development of effective therapies. To address this, a new fly model of AD that genetically produces both human Abeta42 and tau has been created. These "humanized" flies display extracellular deposition of Abeta42, intracellular aggregation of pathological tau, and robust neurodegeneration. The robust pathology of these flies provides an ideal platform to conduct a large-scale identification of genes that can suppress Abeta42+tau neurotoxicity. Therefore, Abeta42+tau flies will be crossed with ~6,500 strains engineered to specifically silence individual fly genes that are also present in humans. First, a primary screen in the fly eye will be performed, which provides a fast-visual result of the effect of silencing every gene. Then, validation of the identified suppressors for behavioral functions, preservation of brain neurons, and development of pathological markers will be performed. It is anticipated that this experimental approach will uncover critical/novel targets for intervention not available to classical experimental models. Thus, the first large-scale attempt at discovering Abeta42+tau suppressors will not only provide information about disease mechanisms but also identify relevant therapeutic targets to approach this overwhelming disorder. This fits perfectly with the mission of the Ed and Ethel Moore Alzheimer's disease Research Program to "stimulate the discovery and validation of a broad spectrum of potential therapeutic targets for AD".

13. **Grant #7AZ13:** CK1 Delta Inhibition To Reduce Sundowning In Alzheimer's Disease

Principal Investigator: Danielle Gulick, PhD

Organization: University of South Florida

Abstract of Proposed Research: Alzheimer's disease is a progressive, devastating form of dementia that affects not only patients but, also, their caregivers, diminishing quality of life for everyone touched by the disease. Although a number of therapeutics are under study, no definitive treatment has been identified. Furthermore, many patients with Alzheimer's disease also struggle with sundowning syndrome, an increase in confusion, agitation, wandering, and aggression during the late afternoon and evening

hours. This syndrome results from a loss of the internal clock that normally sets our daily circadian rhythms, and it is proposed that it can be treated with drugs that will reset the internal clock. To this end, two mouse models of sundowning syndrome will be used to test whether treatment with a drug that resets the circadian clock is sufficient to reduce the symptoms of sundowning. Thus, it is proposed that treating these models, as well as healthy controls, with an inhibitor of casein kinase 1, a key enzyme in the clock. This lab has shown that this inhibitor stabilizes the clock and improves cognition. During drug treatment, it will be assessed whether circadian rhythms are corrected by analyzing home-cage activity in the mice. In separate groups of mice, analysis of whether the drug is able to improve cognition, reduce anxiety, and improve socialization will be performed. These behaviors at four points in the day, every six hours, will be examined to determine whether the changes in behavior are due to a global improvement in function, or to a shift in the time when symptoms are at their worst. In addition, because casein kinase 1 plays a role in the formation of the toxic beta-amyloid peptide that leads to neurodegeneration in Alzheimer's disease, levels of this peptide in mice treated with the inhibitor compared to control mice will be examined. This work will provide a foundation for drug development to improve the lives of patients with Alzheimer's disease and their caregivers by reducing some of the most severe symptoms of the disease.

14. **Grant #7AZ14:** CK1 Delta Inhibition To Reduce Sundowning In Alzheimer's Disease

    Principal Investigator: Rosie E. Curiel, PhD

    Organization: University of Miami School of Medicine

    Abstract of Proposed Research: With the rapidly aging population, early detection of cognitive decline in individuals at risk for Alzheimer’s disease (AD) is a global priority. It is now well-established knowledge that pathological changes occur in the brain decades before the onset of any detectable clinical symptoms. This understanding has shifted the priority in the field from clinical diagnosis and treatment, toward the aim of developing early targeted interventions and pre-symptomatic neuroprotective therapies. For these strategies to be optimally effective and successful, it is critical to accurately identify and target individuals at risk. This has led to a growing emphasis on discovering biological markers that may signal the emergence of preclinical AD states, such as Mild Cognitive Impairment (MCI), and highlighted the importance of capturing very subtle cognitive changes that transpire early in the disease course. Detecting cognitive changes are critical because cognitive changes are used to detect and track disease progression over time from MCI to early AD. In addition, a meaningful change in cognitive status represents a measurable clinical outcome. Traditional and widely used assessment paradigms such as delayed recall and rate of forgetting are not well suited to identify the subtle changes in cognition that manifest during the preclinical stages of AD and early MCI. In addition, they lack cross-cultural applicability, are lengthy, labor-intensive, vulnerable to human error, and associated with practice effects. To this end, the use of computerized testing batteries among older adults have been explored as a more suitable option to mitigate some of the above-mentioned limitations by increasing accessibility to distant sites, promoting efficiency, providing real-time data entry, and

increasing the accuracy of recording responses and response time. However, a major problem with existing traditional computerized batteries is that they are automated versions of traditional neuropsychological tests that lack sensitivity to detect AD-related cognitive decline, and employ the same paradigms originally developed for the assessment of dementia or traumatic brain injury. Measures for early detection of cognitive impairment of Hispanic and non-Hispanic elderly persons that are, both, sensitive and portable, are in increasing demand as it is recognized that early diagnosis is the key to more effective intervention strategies. It is believed that the proposed work is positioned to be at the forefront of this critical area. Three novel computerized tests will be administered to 120 older adults (40 normal elderly, 40 amnestic mild cognitive impairment: [aMCI] and 40 Preclinical AD participants). Half of these subjects will be primary Spanish-speakers while the other half will be primary English speakers. Test – retest reliabilities for the experimental measures will be obtained and the discriminative validity of the instrument will also be examined and compared to traditional memory measures. This project is expected to provide critical data that parallels a recently submitted strong early career R01 grant application to the National Institutes of Health, which will examine changes in cognitive performance using these instruments as they relate to longitudinal biological changes within the brain.

15. **Grant #7AZ15:** Identification Of Functional Regulatory Variants At Alzheimer's Disease Loci

    Principal Investigator: Mariet Allen, PhD

    Organization: Mayo Clinic Jacksonville

    Abstract of Proposed Research: Alzheimer's disease (AD), is the most common form of dementia affecting the elderly, and is known to have a substantial genetic component. Identifying genetic variants that influence disease risk has led to improved understanding of the pathological processes involved in this disease and can greatly inform future research and therapeutic approaches. Furthermore, genetic risk factors and their expressed transcripts and proteins represent potential biomarkers for predicting disease risk and identifying subsets of individuals for targeted clinical treatment or prevention trials. Genome-wide associations studies (GWAS), have identified more than 20 common genetic variants that influence risk for AD. This lab, and others, have shown that some of these variants also associate with expression levels of near-by genes. Importantly these findings implicate the biological mechanism of action (regulation of gene expression) and the likely influenced gene(s). However, GWAS are limited, in that the variants genotyped are largely thought to represent a locus (genomic region), rather than actual functional variants. Additional studies are needed, to fine-map the implicated loci and identify and validate the functional genetic risk variants. This proposal aims to address this knowledge gap by identifying, and annotating, regulatory variants at AD risk loci nominated by disease GWAS. Identifying these variants will reveal the biological basis for the disease risk association at these loci, provide novel insights into the pathophysiology of AD, and generate new leads for therapeutic strategies aimed at treating or curing this disease. Specifically, targeted next-generation sequencing will be used to identify variants that fall within the genetic locus tagged by the common variant(s) and any distal regulatory regions nominated by bioinformatics tools. Importantly, sequencing of subjects that were part of our published work that implicated transcriptional regulation as the likely mechanism at these loci and in which gene expression measures already exist. All identified variants will be evaluated for association with

expression of genes within the locus using these existing expression measures. Additional resources such as variant annotation tools (Computer Assisted Drug Design, Regulome Database, and HaploReg Database) and available regulatory element annotation, will be used to further refine our selection of putative functional variants. Results from available data generated by the Alzheimer's disease sequencing project (ADSP) and the International Genomics of Alzheimer's Project (IGAP) will be used to evaluate the association of the nominated variants with AD risk, where possible. Nominated variants will be assessed in additional samples to confirm the association with gene expression measures, and finally tested in a cell based model, using reporter assays, to confirm the functional impact on gene expression. The expected outcome of the proposed work is the identification of functional regulatory variants at some of the known AD risk loci, which may provide novel insights into the pathophysiology of this disease and nominate therapeutic targets. The identified variants and influenced genes may also represent novel biomarkers for disease risk prediction, critical for design of successful therapeutic trials.

16. **Grant #7AZ16:** Yeast Surface Display Engineering Of Human Fibronectin Domains For Enhanced Brain Delivery Of Alzheimer's Disease Therapeutics

    Principal Investigator: Pete Heinzelman, PhD

    Organization: Mayo Clinic Jacksonville

    Abstract of Proposed Research: More than ninety-five percent of potential Alzheimer's disease (AD) therapeutics and prophylactics have little or no ability to migrate from circulation to brain tissue. These blood-to brain transport limitations necessitate unfeasibly high doses of systemically administered drug to realize beneficial effects within the brain and/or promote off-target side effects throughout the body and thus prevent such transport-impaired molecules from being viable AD drug candidates. Developing a generalizable delivery technology that, both, targets transport-impaired drugs to the blood brain barrier (BBB), a tightly packed layer of endothelial cells surrounding the nutrient supplying blood vessels that radiate throughout the brain, and that facilitates transport of these drugs across the BBB, dramatically expanding this inventory of effective AD pharmaceuticals. This will transform the way clinicians seek to treat and prevent AD by providing the breadth of options needed to enable development of personalized AD treatment and prevention programs through evaluation of patient responses to different drug combinations. Conjugation of small molecule drug-loaded liposomes or protein drugs to 'Trojan Horse' antibodies that, both, bind to proteins and are transported across the BBB, is currently the most utilized strategy for targeting AD drugs to the central nervous system (CNS). Such Trojan Horse antibodies, however, bind to proteins expressed on the BBB, in addition to, many other endothelial tissues throughout the body. This ubiquitous expression results in less than one percent of systemically injected Trojan Horse antibody-drug conjugate doses reaching the brain. This research will address the above implied need for step change improvements in AD drug delivery by simultaneously identifying proteins and/or protein structural features that are specific to or highly enriched on BBB endothelial cells and generating human fibronectin domains (Fn3s), antibody-like biomolecule-binding proteins that are less expensive than antibodies to produce, that bind to these BBB-specific molecular entities and can be superior substitutes for existing Trojan Horse antibodies in targeting AD drugs to the CNS. Adaptation of microscale filtration techniques employed in household cleaner manufacturing to convert BBB endothelial cells into water soluble nanometer-

sized vesicles, known as CytoBits, is the key innovation allowing engineering of highly specific BBB-binding Fn3s. Unlike whole cells, CytoBits are compatible with high-throughput screening methods that utilize magnetic-microspheres and flow cytometry, a microfluidics and fluorescence measurement assisted technique for high fidelity isolation of single yeast cells from populations numbering in the millions, that underlie yeast's surface. This technique display's its power as a technology platform for engineering proteins with binding properties that are well-suited to specific biomedical applications. In this work, yeast display will be utilized to isolate a collection of between twenty-five and fifty BBB-specific Fn3s from a library containing 250 million members; this substantial library size brings strength of numbers to addressing the BBB-binding specificity challenge by each member's Fn3 possessing unique biomolecular-binding properties. This brain-targeted, drug-carrying Fn3s will offer exciting potential to make AD treatment and prevention program personalization a reality.

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

    Principal Investigator: Nilufer Ertekin-Taner, MD, PhD

    Organization: Mayo Clinic Jacksonville

    Abstract of Proposed Research: This proposal entitled "Florida Consortium for African-American Alzheimer's Disease Studies (FCA3DS)" stems from this team's highly successful prior study funded by the same mechanism. The current proposal will leverage the infrastructure and collaborations previously established during the initial grant (5AZ03, 01/12/2015-6/30/2015). The main motivation of this proposal is to enhance Alzheimer's disease (AD) research in African-Americans, which remain an understudied population despite being afflicted by this condition twice as frequently as whites. This team's ongoing and proposed research aims to overcome this knowledge gap, because studying diverse populations with distinct risk profiles is critical to the discovery of a wider array of both genetic and non-genetic risk factors for AD. Such discoveries are essential for the identification of drug targets, preventative measures and healthcare policies aimed at curing or delaying progression of AD, which is especially germane to high risk populations, like African-Americans. During the 5-month course of the prior grant, significant progress was made pertaining to a) sample collections (establishment of IRB approvals, streamlined sample and data collection protocols, training of personnel for sample handling at all sites); b) data generation (generation and quality control of whole exome sequence=WES data on 137 AD and 113 control subjects); and c) data management (generation of the relational FCA3DS database and importing of data into this database). During the following 6-month no-cost extension (7/1/2015-12/31/2015), all the known early-onset AD (EOAD) and late onset AD (LOAD) genes were screened and identified novel genetic variants in African-Americans different than those reported for whites. Specifically, risk variants were discovered in the ABCA7 gene that occurs at a higher frequency in African-American AD subjects. Further, additional variants were detected in two other genes (ZCWPW1, NME8) that showed association with memory scores in this population. Finally, two variants were identified in the EOAD genes PSEN1 and PSEN2. These findings are currently under review (LOAD) and in preparation (EOAD) for submission. Hence, the data that was generated under the prior Florida Health grant highlights the critical importance of studying diverse populations, underscores the potential of our approach and this team's ability to execute

these studies. In the new proposal, the aims are to: 1) Expand the cohort for WES of additional samples; 2) Launch studies of gene expression pathways utilizing blood RNA samples; 3) Utilize plasma amyloid ß and cognition as biomarkers for novel gene/pathway identification. This consortium grant includes three Florida institutions: Mayo Clinic, University of Florida and Mount Sinai Medical Center. Expected outcomes are: 1) Establishment of a sizable African-American cohort with DNA sequence, gene expression, plasma amyloid ß data; 2) Targeted gene expression studies correlated with genetic and clinical outcomes. 3) Identification of novel genes/pathways implicated in AD risk, amyloid metabolism and cognition. This proposal is innovative in that AD gene/pathway discovery studies that utilize combined genetic /expression /protein /cognition data are unprecedented in African-Americans. Expected outcomes of this proposal include a unique resource and impactful pathophysiologic findings in this understudied population.

18. **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

    Abstract of Proposed Research: This is an exciting study that examines amyloid load in the brain as it relates to the performance of novel cognitive stress tests designed to assess vulnerability to proactive semantic interference (PSI) or failure to recover from PSI to brain amyloid load, in two different ethnic and cultural groups of elderly participants (African-American and Hispanic). This data is essential in establishing the utility of novel cognitive stress tests in epidemiological and clinical studies. The proposed investigation is, both, an innovative, as well as, a critical study regarding the relationship between total and regional brain amyloid load and performance on both novel cognitive stress test measures among at risk African-American and Hispanic and White noncommunity-dwelling elders. The validation of cognitive stress tests against biological measures in different ethnic and cultural groups are critical for future epidemiological and clinical research in Alzheimer's disease and related disorders. This proposed work is a natural offshoot of a previously funded Ed and Ethel Moore State of Florida Grant (Loewenstein, PI) and an ongoing NIH longitudinal study (Loewenstein, Principal Investigator). In an important recent paper by Loewenstein et al., (2016) supported by the Ed and Ethel Moore Foundation, it was demonstrated that vulnerability to recovery from proactive interference, based on a novel cognitive stress test, could successfully distinguish between individuals with mild cognitive impairment (MCI), PreMCI (evidence of a history of cognitive decline but normal neuropsychological test results), subjective memory disorder and cognitively normal elders. A critical finding was that among a group of community dwelling elders with PreMCI, subjective memory disorder and no memory complaints (all of these groups that normal scores on traditional 5 of 6 neuropsychological tests), the failure to recover from proactive semantic interference was highly associated with brain amyloid load (an indication of accumulating fibrillar brain amyloid and a high risk factor for Alzheimer's Disease (AD) with $r=-.62$ ($p<.01$) for the precuneus and for the whole brain and ($r=-.60$ ($p<.01$). This exciting finding among predominantly white non-Hispanic elderly indicated that early deposits of brain amyloid in community-dwelling elders were highly related to an early cognitive-behavioral marker (inability to recover from proactive semantic interference). In the current proposal,

existing resources will be leveraged from our ongoing R01 National Institutes of Health (NIH) study (that does not currently include measures of amyloid load) to provide 60 subjects who are well characterized as having mild cognitive impairment (MCI) or PreMCI (subjective memory impairment and clinical evidence of mild decline but neuropsychologically normal). All subjects will have Magnetic Resonance Imaging (MRI) scans of the brain. To study 30 African-American (AA) and 30 Hispanic older adults 60+ years who meet these criteria from a cohort of over 250 potential participants in the NIH study was proposed.

19. **Grant #7AZ19:** Functionalized Intrabodies As Potential Anti-Tau Therapy

    Principal Investigator: Yona Levites, PhD

    Organization: University of Florida

    Abstract of Proposed Research: To rapidly and cost-effectively evaluate potential modifiers of Alzheimer's disease (AD) pathology in mouse models, a "somatic brain transgenics" paradigm was developed, through the delivery of gene constructs packaged into adeno-associated viral vectors and, then, injected into the cerebral ventricles of P0 mice. The mechanisms underlying the abnormal phosphorylation and accumulation of Tau in AD remain unclear, but one of the possibilities is that it might be due to conformational changes in tau in the diseased brain. Anti-tau immunotherapy has recently emerged as a promising approach to target tau, but many mechanistic questions regarding the optimal form of anti-tau immunotherapy remain open. This lab has demonstrated that intracellularly expressed anti-tau intrabodies prevent Tau toxicity and formation of neurofibrillary tangles, and prolonged life span of transgenic mice. It is hypothesized that anti-Tau immunotherapy can be optimized by targeting Tau to proteasomal degradation, cellular machinery that is geared to process and eliminate unneeded or damaged proteins by proteolysis. Preliminary data suggests that Tau aggregation functional intrabodies in cell culture models successfully prevented accumulation of aggregated phosopho-tau. It is proposed to further develop functionalized anti-Tau intrabodies and evaluate them in vivo in Tau transgenic mouse models. Possible mechanism of action of these functionalized intrabodies will also be examined. These studies will provide critical insights into i) whether targeting tau to proteasomal degradation is more efficacious and ii) whether this approach can be utilized toward other neurodegenerative diseases involving misfolded proteins.

20. **Grant #7AZ20:** The Role Of TTC3 In Alzheimer's Disease Pathogenesis

    Principal Investigator: Holly N. Cukier, PhD

    Organization: University of Miami

    Abstract of Proposed Research: Alzheimer's disease (AD) is the most common form of dementia in the elderly. Over 5 million individuals in the United States currently have AD and, as the average age of the population rises, so does the incidence of AD. Genetics plays an integral role in AD risk, but the mechanisms which trigger disease on a cellular level are still undergoing investigation. We recently identified a mutation in the

Tetratricopeptide Repeat Domain 3 gene (TTC3) in 11 relatives diagnosed with AD. This rare DNA change is predicted to be damaging by five distinct computer models. Furthermore, evidence from other researchers have found that brains from deceased AD individuals had lower levels of TTC3 and that the gene is involved in neuronal growth. Therefore, it has been suggested that TTC3 could play a protective role against AD and that genetic changes which reduce TTC3 expression may contribute to AD risk. A series of experiments to better understand the effects of the genetic change that this lab identified by studying induced pluripotent stem cells (iPSC) created from three individuals with the TTC3 change and three non-demented controls without the TTC3 alteration was proposed. iPSCs can be differentiated into disease relevant cell types to recapitulate the disease progression. Genetic tools will, then, be used to introduce the single base pair TTC3 change into the control lines and, in a reciprocal experiment, revert the change back to normal in the AD cases. Both, the original and edited versions of all the stem cell lines, will be grown under conditions that induce them to become neurons, a relevant cell type to study AD. These neurons will be evaluated as they age for morphological changes in shape and connectivity, as well as, being tested for cellular changes in proteins related to AD including amyloid beta and tau. Lastly, RNA will be collected from the cells, in-depth sequencing will be performed and alterations in the regulation of other genes will be looked for. Brain tissue from four relatives with the same TTC3 alteration will be used for RNA sequencing to try to identify RNA changes at both early and late stages of disease. These results will be compared to hundreds of RNA profiles from unrelated AD cases and controls. Through these experiments, the aim is to determine if the TTC3 change results in AD specific consequences in neuronal cells and if correcting this genetic change can fix the cellular abnormalities. The hope is to reveal the role that TTC3 plays in AD pathology, as well as, gain a greater insight into how TTC3 acts in a similar or distinct manner from other causes of AD.

21. **Grant #7AZ21:** Evaluating The Mechanism By Which Tau A152t Modulates Risk Of Tauopathy

    Principal Investigator: Casey Cook, PhD

    Organization: Mayo Clinic Jacksonville

    Abstract of Proposed Research: Aggregation of the tau protein is a neuropathological hallmark of several neurodegenerative disorders classified as tauopathies, including Alzheimer's disease (AD). While mutations in the tau gene microtubule-associated protein tau (MAPT) are known to cause primary tauopathies, no MAPT mutations were linked to AD until the discovery of the A152T gene mutation, which acts as a risk factor for AD. In addition to modulating risk for AD, the A152T tau mutation also influences risk for dementia with Lewy bodies (DLB) and the spectrum of frontotemporal dementia disorders, including progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD). Therefore, understanding how the A152T mutation increases disease risk and identifying new genetic modifiers that impact the resulting phenotype in

A152T mutation carriers could provide significant insight into the pathogenic role of tau in neurodegeneration. Compelling evidence that the A152T variant is associated with increased soluble hyperphosphorylated tau in human postmortem tissue from A152T carriers compared to noncarriers when controlling for disease severity has been collected. Consistent with this, expression of A152T-AAV in nontransgenic mice leads to increased accumulation of hyperphosphorylated tau species that also remains within the soluble fraction. Therefore, it is speculated that the A152T tau variant increases risk of tauopathy by modulating both tau hyperphosphorylation and solubility. Therefore, the current project will investigate the pattern of phospho-tau deposition throughout the brain in A152T carriers and noncarriers to determine how its presence coincides with neurodegeneration. In addition, it will be determined whether phosphorylation of tau is required for the toxicity of A152T in vivo. It is anticipated that by furthering the understanding of how A152T influences risk of tauopathy, the proposed studies will provide novel insight into mechanisms of tau toxicity in AD and other disorders.

22. **Grant #7AZ22:** APOE And Cerebrovascular Aging In Alzheimer's Disease

Principal Investigator: Takahisa Kanekiyo, MD, PhD

Organization: Mayo Clinic Jacksonville

Abstract of Proposed Research: Brain vessels play an essential role in maintaining cognitive functions by providing oxygen, nutrition and growth factors from the blood flow and by eliminating toxic molecules such as carbon dioxide from the brain. Alzheimer's disease (AD) is the most common type of dementia, which causes progressive memory loss in aged people. Many human studies have shown that brain vascular damage is strongly associated with the increased risk for AD. In fact, approximately 80% of AD patients have some extent of brain vascular injuries. While accumulation, aggregation and deposition of toxic amyloid-β (Aβ) peptides in the brain are key events in the pathogenesis of AD, brain vascular dysregulation is likely to precede the pathological event during the disease development. Since brain vessels critically mediate the elimination of Aβ from the brain, the disturbance of the pathway is predicted to induce brain Aβ accumulation. Impairments of brain blood supply and blood-brain barrier (BBB) integrity also cause neuronal damage, synaptic dysfunction, and white matter injuries, which eventually lead to the pathogenic condition referred to as vascular cognitive impairment and dementia. Importantly, aging is a critical factor that contributes to both brain vascular dysregulation and AD pathogenesis. Thus, the major goal of this project is to define molecular mechanisms underlying the relationship between aging and brain vascular dysfunctions using, both, cell and animal models, to explore the pathogenic pathways of AD. In general, aging is predicted to be caused by accumulation of senescent cells in the body. The increase of p16INK4a, which plays an important role in cell cycle regulation, is one of the central mechanisms triggering senescent phenotypes. Therefore, it is hypothesized that aging-related upregulation of p16INK4a in vascular cells disturbs the homeostasis of the brain vascular system and Aβ clearance resulting in AD development. Humans have three types of the apolipoprotein E (apoE) gene (APOE2, APOE3 and APOE4). APOE2 is protective against AD, but APOE4 is the strongest genetic

risk factor for the disease. APOE genotypes are also critically involved in the compromised cognitive performance seen in the elderly, which includes mild cognitive impairment and vascular cognitive impairment. Furthermore, APOE4 also causes dysfunction of brain vascular system, including BBB breakdown and the reduction of small vessels. Thus, our proposal will have an emphasis on the effects of APOE4 on senescent phenotypes caused by p16INK4a induction in the brain vessels. To reach the stated goals, three specific aims are proposed. In aim 1, the impact of senescence and apoE isoforms on vascular cell properties and Aβ metabolism will be determined. In aim 2, senescence- and apoE isoform-regulated cell type-specific pathways in brain vascular pericytes and endothelial cells will be defined. In aim 3, the examination of how the induction of p16INK4a in vascular mural cells and endothelial cells alter the amyloid pathology, the cerebrovascular system and the cognitive functions, depending on apoE isoforms. Collectively, these studies should provide novel insights into the cellular and molecular mechanisms that underlie the contribution of apoE and cerebrovascular aging to AD pathogenesis.

23. **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

    Abstract of Proposed Research: Some drugs have been used in the palliative care of Alzheimer’s disease (AD) to treat some of the symptoms such as depression, anxiety and difficulty sleeping. However, these drugs may cause drug-drug interactions (DDIs). Recently, clinical studies showed that the AD patients are at an increased risk of DDIs. For example, combining cholinesterase inhibitors (such as tacrine, donepezil, galantamine, and rivastigmine) with some drugs could increase the risk of gastrointestinal disorders, bradycardia and loss of consciousness. In addition, an elderly patient with AD may have several medical conditions. The concurrent use of multiple drugs for other diseases among the AD patients has tremendously increased. The presence of multiple diseases may also impair the metabolism in elderly individuals, resulting in DDIs that are not common in healthy individuals. DDIs may have potentially life-threatening outcomes, especially for elderly patients. Therefore, AD patients should carefully evaluate the DDIs when prescription medication is used with other drugs and the detection of DDIs is an important field of AD patients’ healthcare. The Food and Drug Administration (FDA) has routinely collected data on adverse drug events (ADEs) submitted to FDA and stored in the FDA Adverse Event Reporting System (FAERS) since 2004. The availability of real-world data from FAERS provides a rich opportunity to identify unexpected DDIs. However, FAERS contains approximately 7.5 million patient records, making it impossible to manually summarize all these records. Also, DDI information cannot be directly and accurately extracted from reports of patients who receive complex combinations of medications without using appropriate algorithms. It is difficult to identify real DDIs from the huge number of possible combinations of drugs and events. Therefore, in this proposal, the development and evaluation of an efficient computational model that can predict possible DDIs, especially from those records of AD patients in FAERS. The DDIs identified by the computational model will be validated

through a retrospective analysis of electronic health records (EHRs) of AD patients. The mechanism of the DDIs will be explored by using drug-protein, protein-protein networks. The successful completion of this project will provide useful information for doctors to prescribe drugs for the palliative care for AD patients (Focus Area 1.3) more appropriately.

24. **Grant #7AZ24:** Correction Of Tauopathy-Induced Circadian Dysfunction

    Principal Investigator: Joshua Gamsby, PhD

    Organization: University of South Florida

    Abstract of Proposed Research: Sleep is an essential part of a healthy lifestyle. Patients with Alzheimer's disease frequently report having trouble with sleep as part of their illness, which may worsen their other symptoms. However, why Alzheimer's patients have problems sleeping is poorly understood. This proposal is focused on uncovering how Alzheimer's disease impacts the region of the brain that is important for maintaining normal sleep timing, and on improving our understanding of how sleep disruption may contribute to the impaired memory of patients with Alzheimer's disease. It is also proposed that a new approach to treat this often overlooked, but extremely troublesome symptom. This work is hoped to improve the quality of life of patients suffering with Alzheimer's, as well as their caregivers, who must provide for them when they are wakeful.

25. **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

    Abstract of Proposed Research: The accumulation of brain neuronal aggregates comprised of the protein tau is a defining hallmark of Alzheimer’s disease (AD). The abundance and distribution of tau aggregates throughout the brain correlate with AD severity. The direct involvement of tau in disease has been unequivocally established by the discovery of tau mutations that results in progressive dementia. Several recent studies have indicated that the spread of tau aggregates within affected brain regions occurs by cell-to-cell transmission of small amounts of tau aggregates further inducing tau aggregation in neighboring cells. To further inform on the general molecular mechanisms influencing the aggregation and spread of tau pathology, it is proposed to explore the relative effects of wild-type and additional disease-associated mutants in cellular and animal models. Intriguingly, preliminary data generated in this laboratory identified a specific region within tau, which is influenced by several tau mutations, as an important determinant in regulating tau aggregation. The impact of this region and nearby putative tau protein modifications in regulating the aggregation of tau will be

assessed, both, in cellular and animal model systems. Collectively, these studies will 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.

26. **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

    Abstract of Proposed Research: The Alzheimer's Association estimates that someone in the United States of America develops Alzheimer's disease (AD) every 68 seconds and that the rate will increase to every 33 seconds by the year 2050. In Florida alone, the Department of Elder Affairs estimates that about 450,000 people currently live with AD – i.e, approximately 10% of US AD cases are in Florida. These are alarming statistics, since to date, all of the FDA-approved Alzheimer's disease (AD) treatments are palliative, at best, and do not target the main hallmark of the disease, beta-amyloid (Aβ) peptides that aggregate into amyloid plaques in the brain of patients and animal models. There is an enormous need for new therapeutic strategies. One of the drugs that has shown promise to date is resveratrol (RSV). Although it has been investigated for its potential use in AD for more than a decade using cell and animal models, only in December 2015 did a phase II randomized clinical trial present evidence that RSV is indeed beneficial to AD patients. Indeed, this study by the Alzheimer's Disease Cooperative Study group (ADCS) indicated that a high dose of resveratrol (up to 2 grams daily) has beneficial effects including positive alteration of amyloid biomarkers in cerebrospinal fluid. Such high doses are needed because of the poor bioavailability of resveratrol with a marked first pass effect and degradation in the liver. Unfortunately, such high doses will cause gastrointestinal and other dose limiting side effects. A new oral formulation of RSV, JOTROL, has been developed by a Florida-based company (Jupiter Orphan Therapeutics) and shows markedly higher bioavailability when compared to unformulated resveratrol will be studied. It is proposed to test JOTROL in AD animal models to evaluate its efficacy at both preventing and treating AD-like pathology at molecular and behavioral levels. Resveratrol has known epigenetic activity, including activation of the SIRT1 gene in the brain, which is likely to be more pronounced by equimolar doses of JOTROL. This group has successfully used AD animal models to test small epigenetic molecules in the past and anticipate obtaining positive effects with JOTROL in this project. A team of experts with vast experience in Alzheimer's disease, resveratrol chemistry and epigenetics has been assembled.

27. **Grant #7AZ27:** Structure And Toxicity Of Amyloid Beta Hetero-Oligomers

Principal Investigator: Suren A. Tatulian, PhD

Organization: University of Central Florida

Abstract of Proposed Research: Alzheimer's disease (AD) is the major cause of dementia. To date, no effective therapies have been developed for the disease. Identification of novel biomarkers may facilitate development of efficient diagnostic and therapeutic strategies to combat AD. Amyloid beta (Abeta) peptide plays a major role in AD and occurs in various forms. While the most prevalent forms are the 40- and 42-amino acid residue peptides (Abeta1-40 and Abeta1-42), N-terminally truncated and pyroglutamylated Abeta peptides (AbetapE) constitute 10 to 50 % of total Abeta in AD brains, are hypertoxic, and augment Abeta cytotoxicity even at low molar fractions. The molecular mechanism of AbetapE hypertoxicity remains unknown. Currently, Abeta1-42 and Abeta1-40 are the major biomarkers targeted by AD immunotherapy trials, which have led to serious side effects such as meningoencephalitis, vasogenic edema, and brain microhemorrhages. Recently, a monoclonal antibody against AbetapE3-42 has been identified as a promising passive immunotherapy agent in mice. Further efforts towards identification and characterization of novel AD biomarkers, such as hypertoxic Abeta/AbetapE coaggregates, will likely lead to better, clinically acceptable AD immunotherapies. It has been recently identified that Abeta1-42 and AbetapE3-42 reciprocally inhibit fibrillogenesis and shift the aggregation process towards beta-hairpin-like structures stabilized by intramolecular Hydrogen bonding. Cell-based studies showed that Abeta1-42/AbetapE3-42 hetero-oligomers exerted the maximum toxic effect on neuronal PC12 cells as compared to oligomers of individual peptides or fibrils. Collectively, these findings support a novel concept that a) interaction between Abeta1-42 and AbetapE3-42 inhibits fibrillogenesis and promotes formation of hetero-oligomers of unique structure and b) these hetero-oligomers, not Abeta1-42 or AbetapE3-42 oligomers, are the most cytotoxic species and, hence, constitute a novel biomarker to be targeted for efficient AD immunotherapies. Based on these findings, the hypothesis is that heterogeneous aggregates of different Abeta species, including AbetapE, exert the major neurotoxic effect in AD. Hence, the focus of basic and clinical studies should be shifted from individual Abeta species to hetero-oligomers. This project aims at detailed characterization of the structure and cytotoxicity of Abeta/AbetapE hetero-oligomers by pursuing the following specific aims. Aim 1: Identify the effect of AbetapE on Abetafibrillogenesis and accompanying structural transitions upon co-aggregation. The hypothesis that, contrary to the existing paradigm, AbetapE inhibits fibrillogenesis and promotes formation of hetero-oligomers with Abetapeptides will be tested by isotope-edited Fourier Transform Infrared (FTIR) anaylysis, fluorescence, atomic force microscopy and other biophysical and biochemical methods. Aim 2: Determine the critical morphological and atomic-resolution structural distinctions between aggregates formed by AbetapE alone and combined with unmodified Abeta. The hypothesis that AbetapE, Abeta and their mixtures undergo aggregation via distinct structural pathways will be tested at the morphological and atomic levels using advanced transmission electron microscopy and solid state NMR. Aim 3: Determine the cytotoxicities of Abeta, AbetapE, and combined Abeta/AbetapE samples of defined aggregation states and structures. The hypothesis that Abeta/AbetapE hetero-oligomers possess maximum cytotoxicity as compared to Abeta or AbetapE oligomers or fibrillar peptide assemblies will be tested. A wider variety of heterogeneous systems, including other Abeta species such as 40- and 42-residue peptides, will be studied.

APPENDIX B

FISCAL YEAR 2016-2017 ACTIVE GRANTS

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
6AZ01	Mayo Clinic Jacksonville	Murray, Melissa	$ 250,000	$ 81,582.95	$ 168,417.05	1/29/2016	1/31/2018	No	No	No
6AZ02	Florida Atlantic University	Galvin, James	$ 250,000	$ 28,461.67	$ 221,538.33	2/11/2016	1/31/2018	No	No	Yes
6AZ03	University of Miami	Loewenstein, David	$ 88,470	$ 55,360.66	$ 33,109.34	1/28/2016	1/31/2018	No	No	No
6AZ04	University of Miami	Czaja, Sara	$ 249,096	$ 104,323.49	$ 144,772.51	1/20/2016	1/31/2018	No	No	No
6AZ05	University of Florida	Cottler, Linda	$ 250,000	$ 40,623.54	$ 209,376.46	2/23/2016	1/31/2018	No	No	No
6AZ06	Mayo Clinic Jacksonville	Fryer, John	$ 250,000	$ 136,676.86	$ 113,323.14	1/29/2916	1/31/2018	No	Yes	No
6AZ07	University of Florida	Wicklund , Meredith	$ 250,000	$ 93,429.69	$ 156,570.31	2/23/2016	1/31/2018	No	No	No
6AZ08	University of Miami	Wahlestedt, Claes	$ 250,000	$ 171,423.64	$ 78,576.36	1/20/2016	1/31/2018	No	No	No
6AZ09	Florida State University	Terracciano, Antonio	$ 250,000	$ 169,633.59	$ 80,366	2/11/2016	1/31/2018	No	No	No
6AZ10	University of Florida	Horgas, Ann	$ 249,436	$ 52,324.71	$ 197,111.29	2/23/2016	1/31/2018	No	No	No
6AZ11	Florida State University	Meckes, David	$ 81,499	$ 46,143.72	$ 35,355.28	1/29/2016	1/31/2018	No	No	No
6AZ12	Mayo Clinic Jacksonville	Lucas, John	$ 200,000	$ 32,232.01	$ 167,767.99	2/11/2016	1/31/2018	No	No	No
6AZ13	Mayo Clinic Jacksonville	Kim, Jungsu	$ 200,000	$ 132,256.75	$ 67,743.25	1/29/2016	1/31/2018	No	No	No
6AZ14	University of West Florida	Guttman, Rodney	$ 81,499	$ 18,769.71	$ 62,729.29	1/19/2016	1/31/2018	No	No	No
6AZ15	University of Florida	Bowers, Dawn	$ 100,000	$ 34,290.11	$ 65,709.89	2/11/2016	1/31/2018	No	No	No

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

1. **Grant #6AZ01:** Clinicopathologic And Genetic Differences Of Neurodegenerative Health Disparities In The State Of Florida Brain Bank

   Principal Investigator: Melissa Murray, PhD

   Organization: Mayo Clinic Jacksonville

   Progress Report: The initial focus has been to build a database of neuropathologic, clinical and genetic data for the state of Florida brain bank cases. Research has obtained Braak neurofibrillary tangle stage on a total of n=2647/2809, bringing us up to 94% completion, and have obtained Thal amyloid phase on a total of n=2390/2809, bringing it up to 85% completion. Classification of cases as Alzheimer's disease (n=1639), frontotemporal lobar degeneration (n=235), Lewy body disease (n=675) has been completed. Tissue blocks have been prioritized for processing, in which, the first 36 have been cut, stained, and scanned.

   Follow On Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals: None at the time of reporting

   Patents: None at the time of reporting

2. **Grant #6AZ02:** Caring For You (C4U): A Novel Intervention To Improve Caregiver And Patient Outcomes And Quality Of Life

   Principal Investigator: James Galvin, MD, MPH

   Organization: Florida Atlantic University

   Progress Report: Alzheimer's Disease caregiving is a biomedical challenge. As a direct result of caregiving, family care givers (FCGs) are at an increased risk for health problems such as heart disease, headaches, digestive problems, disturbed sleep, reduced immunological function, and inflammatory biomarker changes. These biomedical challenges potentially limit the FCGs' ability to care for themselves and, thus, affect the care of the person with dementia (PWD), often with deleterious and expensive consequences (poor health outcomes, hospital admissions, transition to long-term care). This project tests a novel, bilingual intervention, Caring for You (C4U) [in Spanish "Cuidandote""] in a clinical trial of 150 PWD/FCG dyads compared with a "usual care" control group (i.e., printed information, support groups). The curriculum, study guide, pre/post – tests, instructor training program and related materials are all completed. The social worker was hired and trained to facilitate the groups. The recruitment coordinator has been engaging community centers to discuss the project. Co-investigator, Dr. Christine Williams, has initiated recruitment of our control condition arm from the Florida Atlantic University's Memory and Wellness Center. We recruited the first eight

individuals for our pilot phase intervention. We will use this pilot phase for formative evaluation of all study materials and, then, will finalize the study guide and training program. The new facility opened in July 2016 and clinical operations started in October due to delays in Medicare registration and electronic medical records (eMR) initiation – these were unanticipated delays.

Follow On Funding: Harry Mangurian Foundation $100,000 gift

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

3. **Grant #6AZ03:** Post – Doctoral Fellowship In Neuropsychology Of Pre-Clinical Alzheimer's Disease

   Principal Investigator: David Loewenstein, PhD

   Organization: University of Miami

   Progress Report: There is a pressing need to train promising researchers to study more innovative ways of assessing and diagnosing persons in the earliest stages of Alzheimer's disease (AD). This project presents an unprecedented opportunity for post-doctoral neuropsychology fellowship training to a) study the earliest pre-clinical manifestations of AD; b) participate in the development and implementation of novel measures to assess pre-clinical AD; c) learn to clinically evaluate different ethnic and cultural groups for early stage mild-cognitive impairment; d) learn how to interpret and to conduct research relating cognitive and functional test findings to biological measures of the brain and e) learn to publish papers and prepare National Institutes of Health (NIH) funded applications for further extramural grant support. The post-doctoral fellow, Dr. Allyn Penate, continues to gain invaluable experience working on two large NIH grants where she is developing research skills in the early detection of Alzheimer's disease in different cross cultural groups. She has learned different aspects of neuroimaging data acquisition, preprocessing and analyses, analyzing data regarding novel cognitive test measurers, conducting clinical interviews and helping to maintain databases. Dr. Penate has also been receiving training on novel methods of functional assessment, didactics in data analyses, image analyses, neuro-anatomy, and test development. She supervises graduate students under the supervision of Dr. Loewenstein, as well as, neuropsychologist Dr. Rosie Curiel from the University of Miami. She is also working with Dr. Loewenstein in learning about vulnerability to semantic interference, assessment of prospective memory and amyloid PET scans.

   Follow On Funding: None at the time of reporting

   Collaborations: Mt. Sinai Medical Center is where Dr. Loewenstein has a project which is part of the Florida Alzheimer's Disease Research Center. Dr. Penate works on that project. She works the rest of the time at the University of Miami on Dr. Loewenstein's R01 project.

Journals: None at the time of reporting

Patents: None at the time of reporting

4. **Grant #6AZ04:** A Non-Pharmacological Intervention For Patients With Alzheimer's Disease

   Principal Investigator: Sara J. Czaja, PhD

   Organization: University of Miami

   Progress Report: This study will develop and test the efficacy and feasibility of a dyadic-based intervention program (DT), delivered through state-of-the-art computer tablet technology, that will focus on both the caregiver and the Alzheimer's diseased patient through the combination of an evidenced-based caregiver intervention component and an evidenced-based cognitive/functional training component for the patient. The program is tailored to the needs of the caregiver and emphasizes issues important to caregivers in the earlier stages of caregiving, but also targets issues across the caregiving trajectory to help prepare the caregiver for later stages of the disease. Infrastructure development for this project has resulted in 20 Dell touch-screen laptops, and the assessment battery for the caregiver and the care recipient has been finalized. A stakeholder meeting was completed to identify strategies to raise awareness of Alzheimer's disease in this community and to engage caregivers to participate in research programs. Further refinement of the intervention to expand the degree of integration between the caregiver and care recipient components were also performed. Under development is the caregiver component of the intervention. A position to serve as the participant recruiter is currently being finalized. The intervention content is being revised to increase the integration between the caregiver and care recipient aspects of the intervention. Specifically, focus has increased on the involvement and sharing of pleasant activities and facilitating communication. A grant proposal was submitted to the National Institute of Health to expand this project.

   Follow On Funding: None at the time of reporting

   Collaborations: Mental health counseling students from the University of Miami are working on this project as part of their education/training. The students are required to do a practicum towards the end of their course work. As an outplacement site, the Center on Aging has been very successful in recruiting students to work on an array of research projects. There are two students involved in this project. One of the students has been involved with other caregiving studies at the Center on Aging. In general, the practicum students will be involved in:

   - Recruitment and screening of potential participants
   - Data collection and entry
   - Delivery of intervention sessions under the supervision of Dr. Loewenstein

   Journals: None at the time of reporting

   Patents: None at the time of reporting

5. **Grant #6AZ05:** Linking Older Adults From The Community In Florida To Memory Screening And Related Health Research

   Principal Investigator: Linda B. Cottler, PhD, MPH

   Organization: University of Florida

   Progress Report: This project will raise awareness for Alzheimer's disease in the community and provide ethnically diverse community members an unprecedented opportunity to participate in innovative, culturally relevant screening, treatment efforts and research initiatives. We will do this by engaging community members through our person-centered, evidence based outreach model, HealthStreet, now based in Gainesville and Jacksonville. Research efforts have centered on start-up activities. Necessary forms and protocols used at HealthStreet have been modified as needed and the revisions have been submitted to the University of Florida Institutional Review Board. Geographically, the project coordinator has focused on the Miami area, where community health workers (CHWs) have been hired and trained in all HealthStreet protocols, and added to the Institutional Review Board (IRB) project. A resource guide has been started for those counties, for use by the CHWs in referring community members for needed services, and a list of outreach locations for CHWs is growing. Spanish translations and back-translations are in progress to facilitate increased diversity of recruitment. The first HealthStreet Intake has been completed in Miami-Dade County, and based on this initial work, projects in the other designated counties in Florida will be initiated.

   Follow On Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals: None at the time of reporting

   Patents: None at the time of reporting

6. **Grant #6AZ06:** Clusterin Prevention Of Alzheimer Pathology

   Principal Investigator: John Fryer, PhD

   Organization: Mayo Clinic of Jacksonville

   Progress Report: Two major pathologies are present in Alzheimer's brain: amyloid plaques composed of the amyloid-beta (Aβ) peptide and neurofibrillary tangles composed of hyperphosphorylated forms of the tau protein. A third pathology, often under recognized, that is present in >85% of Alzheimer's brains is the deposition of Aβ in the walls of cerebral vessels, termed cerebral amyloid angiopathy (CAA), that can result in small microhemorrhages and large, recurrent, and frequently fatal lobar hemorrhages. The goal of this project is to determine whether the Clusterin (CLU) protein alters these pathologies using mouse models. At the time of application, we had generated amyloid models with reduction of CLU protein by 50% (CLU+/- mice) on the APP (amyloid plaque pathology)/PS1 (parenchymal space) background ("Bourchelt" model of amyloidosis).

In our previous progress report, we reported a few older mice that completely lack the CLU protein (CLU-/- mice) in the APP/PS1 model and the results are very striking. Normally, this model deposits the majority of the Aβ peptide in brain parenchyma as amyloid plaques with a very small amount depositing in the vessels as CAA. However, the APP;CLU-/- mice show the complete opposite with the majority of the Aβ peptide in brain parenchyma as amyloid plaques with a very small amount depositing in the vessels as CAA. However, the APP;CLU-/- mice show the complete opposite with the majority of Aβ depositing as CAA with almost no parenchymal plaques. Several animals have now been added to this analysis, and the amyloid load in vessels as CAA have been quantified and found that it is significantly increased in CLU-/- mice. It has also been found that, although, CLU-/- mice have ~4 – fold increase in the amount of CAA, they actually have significantly less hemorrhage. These data are in the final stages of manuscript preparation for submission to a high – impact journal.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: Identification of plexin A4 as a novel clusterin receptor links two Alzheimer's disease risk genes. Kang SS, Kurti A, Wojitas A, Baker KE, Liu CC, Kanekiyo T, Deming Y, Cruchaga C, Estus S, Bu G, Fryer JD. Hum Mol Genet. 2016 Jul 4. Pii:ddw188. [Epub ahead of print]
PMID:27378688

Patents: None at the time of reporting

7. **Grant #6AZ07:** Consortium For Diagnostic Algorithm With Novel Marker's In Early Alzheimer's Disease

   Principal Investigator: Meredith Wicklund, MD

   Organization: University of Florida

   Progress Report: A primary goal of this consortium is to study the earliest manifestations of Alzheimer's disease (AD) to develop effective diagnosis tools, possibly leading to more effective treatments. The aim is to develop novel neuropsychological measures, functional assessments and novel imaging techniques for culturally diverse populations that are sensitive to the earliest manifestations of AD. With a sophisticated data repository that allows easy transfer of clinical data across clinical sites, the aim is to develop computerized diagnostic algorithms using multimodal data that will allow for the standardized and sensitive diagnosis of early AD. Approval from the University of Florida Institutional Review Board has been obtained so that subject recruitment may begin. Currently, five subjects have completed the protocol including obtaining neuropsychological data, imaging and genetic data. Additional subjects are scheduled to complete the protocol during the next quarter. A recruitment strategy has been identified and implemented to continue to recruit subjects.

   Consortium members are participating in monthly teleconferences for consensus diagnosis on subjects already recruited through the Alzheimer's Disease Research Centers and subjects recruited through this study will be added to those conferences for

consensus diagnosis. Consortium members and the psychometrist have been trained in administration of the neuropsychological measures. Consortium members with the Clinical and Translational Science Informatics and Technology (CTS – IT) have developed the databank and written the code for the computerized algorithm to compare to consensus diagnosis to aid in standardization of diagnosis of pre-MCI and eMCI. The algorithm is in the final stages of data checking before full implementation.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

8. **Grant #6AZ08:** Epigenetic Modulation Of Alzheimer's Disease Hallmarks

Principal Investigator: Claes Wahlestedt, MD, PhD

Organization: University of Miami

Progress Report: This lab has successfully devised approaches to epigenetically target the hallmarks of Alzheimer's disease (AD). Using small molecules, we can successfully target the gene expression of culprits responsible for the production of amyloid beta (Aβ), as well as, other AD-related proteins. So far, testing continues on the effect of clinical analogs of CTI-309 on gene and protein expression of Alzheimer's Disease (AD) biomarkers. Observation results are encouraging on genes that affect memory. Experiments are in process to confirm those results and possible mechanisms involved. Data are constantly being collected to evaluate the use of these compounds in the context of Alzheimer's disease.

Additionally, we are testing compounds that have been generated by our medicinal chemists in our assays. In vivo work is progressing and data collection has begun. So far, results indicate that some of the compounds have strong effect on AD–related genes, and possibly neuro-inflammation in the brain of treated mice. A cohort of animals are still aging for further testing of compounds.

Follow On Funding: None at the time of reporting

Collaborations: One graduate student and three undergraduate students, all from the University of Miami, are currently receiving training and performing research under this research project.

Journals: None at the time of reporting

Patents: None at the time of reporting

9. **Grant #6AZ09:** Optimization Of "Powerful Tools" For Caregivers Of Dementia Patients

Principal Investigator: Antonio Terracciano, PhD

Organization: Florida State University

Progress Report: The objective of this project is to conduct a clinical trial to evaluate and enhance the clinical translation of a caregiver psychoeducational training. The intervention, Powerful Tools for Caregivers, is a 6-week, scripted educational program for family caregivers implemented in a group setting, led by two trained group leaders. Steady progress is being made toward the recruiting goal of 60 participants; currently, 45 participants are enrolled. Participants have completed 73 assessments, and 10 participants have completed all phases of the study. Data entry is ongoing. To increase recruitment, seniors in Leon County are being recruited as well as surrounding counties that signed up with the FSU Institute for Successful Aging.

Follow On Funding: None at the time of reporting

Collaborations: Florida State University, including one undergraduate and one graduate student

Journals: None at the time of reporting

Patents: None at the time of reporting

10. **Grant #6AZ10:** An Analgesic Trial To Reduce Pain And Behavior Disruptions In Nursing Home Residents

Principal Investigator: Ann Horgas, PhD

Organization: University of Florida

Progress Report: The primary aim of this project is to evaluate the effectiveness of routinely administered acetaminophen (1,000 mg, every 8 hours) in reducing behavioral expressions of dementia (e.g., agitation and aggression) in long-term care residents with moderate-to-severe Alzheimer's disease, due to reduced untreated pain. During this quarter, work has extensively progressed for study implementation. The following provides an overview of other progress: internal University of Florida (UF) regulatory compliance documentation for billing; created RedCap database; completed study folders and data storage procedures; established procedures for laboratory specimen processing; completed training in administration of study measures; and obtained certification in administration of the Clinical Dementia Rating Scale. Development of randomization procedures and study forms to document physician orders to implement the protocol, administration logs for study drugs, and other study requirements have been completed. The negotiation and refinement of procedures with the compounding pharmacy regarding the preparation, labeling, and blinding transfer, storage, administration, documentation, and return of unused study drugs was also furthered. In the process of negotiating procedures with the study site and the pharmacy, it was discovered that medications must be prepared in unit-dosed blister packs for each study participant. The contract was re-negotiated. A budget revision was submitted and approved.

The medical staff and administration of Oak Hammock, a continuing care retirement center in Gainesville, FL, have met to identify study participants, and have begun the recruitment process. During this process, several modifications to the study protocol that are needed, were identified. Revisions have been submitted to the UF Institutional Review Board (IRB); approval is pending. These are minor changes that do not affect the study aims, but that reflect the reality of recruiting people with dementia for this study. The project maintains communication with UF IRB on study modifications. The consultant assisted with establishing manual of operations and recruitment and blinding procedures.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

11. **Grant #6AZ11:** Blood Exosomes And Neurodegenerative Disease

Principal Investigator: David G. Meckes, Jr., PhD

Organization: Florida State University

Progress Report: This study will address the current limitations of exosome-based diagnostics and provide novel strategies for molecular-based epidemiological studies. The objectives for the study are twofold: 1) to develop techniques for identifying tissue origins of circulating exosomes, and 2) to compare and characterize brain-derived exosomes present in human blood samples from healthy, mild cognitively impaired, and AD patients. So far, the collection of brains and blood from our preclinical mouse model of Alzheimer's disease for exosome purification was completed. Six brains (3 Wild Type and 3 Alzheimer's disease) were harvested and up to 4mL of plasma from 2, 4, 6, 8 and 10+ month old mice. In total, we have collected 30 brains and 20 mL of plasma for exosome purification and downstream proteomic and RNA-Seq analyses. Purification of exosomes from the tissue and blood have begun, using the size exclusion chromatography and Optiprep density gradient methods developed in the previous quarter. The purity and yield of exosomes was evaluated by nanoparticle tracking and immunoblot for exosome markers. Exosome samples from the 10 month group were processed for mass spectrometry analyses at the FSU Translational Sciences Laboratory. In addition to the in vitro and mouse experiments, final FSU IRB approval to begin our human studies has been received. Patient education and recruitment began this quarter with Westminster Oaks, a retirement community in Tallahassee, and through the Alzheimer's Project. Our goal is to complete the neurological assessment tests and blood draws over the next quarter.

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 #6AZ12:** Neuropsychological Norms For Ethnically Diverse Florida Elders

Principal Investigator: John Lucas, PhD

Organization: Mayo Clinic Florida

Progress Report: The goal of this project is to collect normative data from a sample of ethnically diverse Florida elders on a brief, standardized neuropsychological test battery adopted by the National Alzheimer's Coordinating Center (NACC) for the Uniform Data Set (UDS 3.0). To date, Mayo Clinic Florida (Jacksonville) has enrolled 55 African American participants. Mt. Sinai Medical Center (MSMC, Miami) has enrolled 54 Spanish-speakers and 54 English-speakers. University of South Florida (USF) obtained Institutional Review Board approval for this study during this reporting quarter but has not enrolled participants. The Neuropsychology division at USF was closed on June 30, 2016, and the principal investigator of the USF site was relocated to the Department of Neurosurgery. This has caused start-up delays at the USF site. In the interim, the Biostatistics team at Mayo Clinic has begun to work with the University of Florida to export data collected at Mt. Sinai to a single, common dataset housed at Mayo Clinic. Once that effort is complete, a mechanism will be set up for data transfer from the USF site to Mayo.

Follow On Funding: None at the time of reporting

Collaborations: MSMC (Miami) is enrolling participants under this protocol. The University of South Florida (Tampa) is expected to begin enrolling participants shortly. The University of Florida (Gainesville) is managing the data collected at MSMC for transfer to the Mayo Clinic Florida database. No students are training or performing research under this protocol.

Journals: None at the time of reporting

Patents: None at the time of reporting

13. **Grant #6AZ13:** Targeting Apoe For Alzheimer's Disease Drug Discovery

Principal Investigator: Kim Jungsu, PhD

Organization: Mayo Clinic Florida

Progress Report: This study regulates low density lipo-protein receptor (LDLR) level in a mouse model of beta-amyloidosis by modulating a novel LDLR-interacting protein. To test this hypothesis, a gene therapy approach will be used to determine whether a novel LDLR-interacting protein will affect amyloid deposition in the brain. In addition, small molecule libraries will be screened to identify lead compounds as potential drug candidates. So far, virtual screening for (IDOL) protein inhibitors was performed using natural products and golden fragment libraries. Molecular docking approach was utilized to identify drug candidate compounds. NatX library is a commercially available natural products library of over 60,000 compounds. Golden fragment library is a commercially available library of chemotype fragments widely used in medicinal chemistry design. The top hit compound, NATX16401, binds with (IDOL) protein in a region common to the consensus sequence peptide. The NatX16233 compound binds with (IDOL) protein in a region common to the consensus sequence peptide and is ranked second among the top 150 predicted virtual screening hits. The NatX23939 compounds binds with (IDOL) protein in region common to the consensus sequence peptide and is ranked third among the top 140 predicted virtually screened (VS) hits.

Follow On Funding: None at the time of reporting

Collaborations: There has been no postsecondary educational institution directly supported by this grant. An associate student graduated from Florida State College at Jacksonville (FSCJ) was hired, and the plan is to train her in this lab for her career development.

Journals: None at the time of reporting

Patents: None at the time of reporting

14. **Grant #6AZ14:** Enhancing Detection Of Alzheimer's Disease Biomarkers Using Phage-Derived Quantification (Pdq)

    Principal Investigator: Rodney Guttmann, PhD

    Organization: University of West Florida

    Progress Report: This project will address a major barrier to the early diagnosis of Alzheimer's disease and related disorders through the development of a highly sensitive and low-cost approach to detecting disease-relevant tau metabolites. The goal is to develop a phage-based method and quantification platform (PdQ) to increase sensitivity of detection for low-abundance tau forms that may be present in blood or other easily accessible biofluids. So far, the production of tau protein has ramped up utilizing a new procedure that has resulted in excellent purity. Utilizing this protein, a new approach has been added to create phage that recognize tau by blotting and are currently completing the final round of planning. The rationale for this added approach was the realization that

in addition to ELISA approaches for testing tau, western blotting is also an efficient and widely used method. In addition, using the blotting method combined with the sonication method by Mojca Lunder et al, elution is more effective. Improved elution of phage was also observed. Using this method, excellent enrichment phage from the PHD 12mer library after two rounds was obtained, and the final round and comparison to Tau-5 will be completed in the next quarter.

Progress has also been made in developing a robust qPCR approach. During this period, primers were designed and preliminary testing has been conducted using our qPCR approach for high sensitivity detection. Optimized conditions are expected, and a comparison of sensitivity to colorimetric and the phage plaque method will be initiated during the next quarter.

The research team has begun further characterization of the pilot data phage for comparison with Tau-5 by ELISA. This aspect has primarily been the responsibility of the principal investigator. Initial comparisons are expected to be completed using standard ELISA methods in the next quarter. With the successful optimization of the qPCR protocol, data are expected to be reported in January.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

15. **Grant #6AZ15:** Pilot Intervention In Mild Cognitive Impairment: A Proof Of Concept Study With NIR

Principal Investigator: Dawn Bowers, PhD

Organization: University of Florida

Progress Report: This project tests a non-invasive, low risk and low cost brain stimulation approach to enhancing cognition and mood in individuals with mild cognitive impairment, who are at a high risk of transitioning to Alzheimer's disease. Participants have been identified, screened, and enrolled in our intervention trial. Four participants have completed the two-week Near Infrared (NIR) intervention; another four participants are currently enrolled and partially through the two-week intervention. Also, pre-post imaging was analyzed from a subset of participants who underwent Functional Magnetic Resonance Imaging (fMRI) and Magnetic Resonance Spectroscopy (MRS) scans, before and after the two-week intervention. Dr. Adam Woods is analyzing the neuroimaging data (MRS-1HP spectroscopy). Initial analyses suggest significant changes in one of the ATP peaks following intervention, with the changes occurring over

the frontal but not temporal region. This is promising, but will need to see if these initial findings continue to hold with additional participants. Additional potential participants, who have expressed interest, have been secured, and participant recruitment will continue.

Follow On Funding: None at the time of reporting

Collaborations: Dr. Preeti Sinha, MD, a geriatric psychiatrist, from the National Institute of Mental Health and Neuroscience (NIMHANS) of Bangalore, India, has joined our team. She is a Fogarty Fellow, through a grant that is directed by Dr. Cotter (principal investigator) at the University of Florida. Dr. Sinha is spending six months in this laboratory and is hoping to extend her stay through the Fogarty Fellowship for another six months.

Dr. Sinha has designed a dose-response relationship study to determine which of two doses of NIR stimulation is better for inducing neuroimaging changes as measured via ATP on MRS spectroscopy and by changes in functional connectivity. This study, entitled “Dose Response relationship between Near Infrared (NIR) Light Stimulation and Functional Brain Activity” (Institutional Review Board #2016014040) just received conditional approval by the UF Institutional Review Board on October 5, 2016 with some contingencies which we are now addressing. Dr. Sinha hopes to begin data collection later in the fall. Her second project is still under IRB review.

Journals: None at the time of reporting

Patents: None at the time of reporting

APPENDIX C

FISCAL YEAR 2016-2017 CLOSED GRANTS, Funding Fiscal Year 2014-15

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditures	Unspent	Executed Date	End Date	Patents	Publications	Follow-on Funding
5AZ01	Mayo Clinic Florida	Rademakers, Rosa	$ 500,000	$ 500,000.00	$ 0	1/22/2015	12/31/2015	No	Yes	Yes
5AZ02	University of Miami	Loewenstein, David	$ 500,000	$ 498,233.10	$ 1,766.90	12/29/2014	12/31/2015	No	Yes	Yes
5AZ03	Mayo Clinic Florida	Ertekin-Taner, Nilufer	$ 500,000	$ 500,000.00	$ 0	1/12/2015	12/31/2015	No	Yes	Yes
5AZ04	University of Florida	Lewis, Jada	$ 250,000	$ 248,999.08	$ 1,000.92	1/20/2015	12/31/2015	No	No	No
5AZ05	University of Florida	Dore, Sylvain	$ 225,000	$ 201,170.27	$ 23,829.73	12/29/2014	12/31/2015	No	No	No
5AZ06	University of Miami	Moraes, Carlos	$ 200,000	$ 198,775.23	$ 1,224.77	12/29/2014	6/30/2015	No	Yes	No
5AZ07	University of South Florida	Kang, David	$ 200,000	$ 199,508.87	$ 491.13	1/20/2015	12/31/2015	No	Yes	No
5AZ08	Mayo Clinic Florida	Bu, Guojun	$ 200,000	$ 200,000.00	$ 0	1/23/2015	6/30/2015	No	Yes	Yes
5AZ09	University of Miami	Wahlestedt, Claes	$ 200,000	$ 199,949.12	$ 50.88	12/29/2014	6/30/2015	No	Yes	No
5AZ10	University of South Florida	Tan, Jun	$ 112,500	$ 112,167.29	$ 332.71	12/29/2014	12/31/2015	Yes	No	Yes
5AZ11	University of South Florida	Lee, Daniel	$ 112,500	$ 102,197.36	$ 10,302.64	1/20/2015	12/31/2015	Yes	No	Yes

CLOSED GRANTS FISCAL YEAR 2016-2017

(Funding fiscal year 2014-2015)

1. **Grant #5AZ01:** Identification Of Novel Alzheimer's Disease Genes And Disease Associated Pathways Through Fpads: A Florida Presenile Alzheimer's Disease Subjects Registry

   Principal Investigator: Rosa Rademakers, PhD

   Organization: Mayo Clinic Florida

   Progress Report: Major achievements were as follows: 1) creation of FPADS presenile Alzheimer's disease (AD) patient registry, including obtaining IRB approval at all five sites and initiation of first patient recruitment, including 2 skin fibroblasts; 2) mutation analysis of known genes in existing Mayo Clinic early-onset AD cohort and preparation of deoxyribonucleic acid (DNA) of high quality for 250 patients without mutations for whole genome sequencing; and 3) cloning of all 12 kDa transmembrane protein (DAP12) mutants into expression vectors. The most important findings were the identification of a founder mutation in Presenilin1 (p.G206A) in 19 Hispanic Floridian AD patients.

   Mutation analysis of more than 600 early-onset AD patients was completed with 52 mutation carriers identified. Whole-genome sequence data obtained from 250 early-onset AD patients; data was aligned and annotated and analysis started. Cloning of 4-diaminopyridine (4-DAP) mutants was completed and effect on DAP12 and Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) determined in cell culture. Genetic case-control association analysis of DAP12 was completed (significant association identified; p=0.0012).

   A total of 55 early-onset AD patients were ascertained, including four patients with skin biopsy since inception of grant. Completed alignment and annotation and upload into Gem.app database of 250 whole genome sequences from 250 patients. Initial analysis of this dataset for mutations in known neurodegenerative disease genes resulted with exciting findings for Sortilin-Related Receptor (SORL1) and TREM2. Analysis continued of the p.G206A Hispanic Early Onset Alzheimer's disease mutation including the study of genetic modifiers of age at onset in this population. The paper describing this mutation in our cohort is now accepted in American Journal of Neurodegenerative Diseases. Continued functional analysis of DAP12 with comprehensive genetic and expression study is now in preparation for Neurology Genetics, an open access journal.

   Follow On Funding: VIAGenetics $120,000

   Collaborations: None at the time of reporting

   Journals:

   - Ravenscroft, T. A., Pottier, C., Murray, M. E., Baker, M., Christopher, E., Levitch, D., Betancourt, A. … & Rademakers, R. (2016). The presenilin 1 p. Gly206Ala mutation is a frequent cause of early-onset Alzheimer's disease in Hispanics in Florida. American journal of neurodegenerative disease, 5(1), 94.

Patents: None at the time of reporting

2. **Grant #5AZ02:** A Consortium To Study Novel Markers Of Early Alzheimer's Disease

   Principal Investigator: David Loewenstein, PhD

   Organization: University of Miami

   Progress Report: The study was initiated, achieved recruitment targets, and successfully transferred clinical and neuropsychological profiles into Spanish and English. Databases were setup at UF with successful data transfer protocols for both clinical and neuroimaging data (e.g., volumetric MRI, amyloid, diffuse water diffusion). Weekly committee and subcommittee meetings were held to discuss progress and ensure uniformity in clinical, neuropsychological and neuroimaging protocol administration and scoring.

   Study objectives were met in under six months by recruiting 30 subjects from Mount Sinai Medical Center (MSMC, Miami) and 15 subjects form the University of Florida (UF). The database was completed in RedCap, along with the corresponding database forms, which allowed for data entry in both English and Spanish. Analysis was completed and resulted in two presentations (1 paper) selected for presentation at the prestigious Alzheimer ’s disease international Conference in July 2015 (Washington, DC) highlighting novel cognitive and biomarker studies.

   A paper, written by Loewenstein et al, was accepted, pending minor revisions, by the American Journal of Geriatric Psychiatry. This demonstrates that the Loewenstein-Acevedo Scale for Semantic Interference and Learning (LASSI-L) cognitive stress tests, particularly the failure to recover from proactive interference, is associated with increased brain amyloid load, even among neuropsychologically normal elders.

   Follow On Funding: National Institutes of Health $7,610,665 2015-2020 (Project 1) and $718,545 for 2015-2020 (Project 2)

   Collaborations: None at the time of reporting

   Journals:

   - Loewenstein DA, Greig MT, Curiel R, et al. Proactive Semantic Interference Is Associated With Total and Regional Abnormal Amyloid Load in Non-Demented Community-Dwelling Elders: A Preliminary Study. The American Journal of Geriatric Psychiatry : official journal of the American Association for Geriatric Psychiatry. 2015;23(12):1276-1279. doi:10.1016/j.jagp.2015.07.009.

     ABSTRACT: High levels of association were present between total amyloid load, regional amyloid levels, and the PSI measure (in the entire sample and a subsample excluding MCI subjects). RSI and other memory measures showed much weaker associations or no associations with total and regional amyloid load. No associations between amyloid levels and non-memory performance were observed. In non-

demented individuals, vulnerability to PSI was highly associated with total and regional beta-amyloid load and may be an early cognitive marker of brain pathology.

- Loewenstein DA, Curiel RE, Greig MT, Bauer RM, Rosado M, Bowers D, Wicklund M, Crocco E, Pontecorvo M, Joshi AD, Rodriguez R, Barker WW, Hidalgo J, Duara R. A Novel Cognitive Stress Test for the Detection of Preclinical Alzheimer Disease: Discriminative Properties and Relation to Amyloid Load. Am J Geriatr Psychiatry. 2016 Oct;24(10):804-13.

  ABSTRACT: LASSI-L deficits were identified among 89% of those with MCI, 47% with PreMCI, 33% with SMI, and 13% classified as CN. CN subjects had no difficulties with recovery from PSI, whereas SMI, preMCI, and MCI participants evidenced deficits in recovery from PSI effects. Among a subgroup of participants with normal scores on traditional neuropsychological tests, the strong associations were between the failure to recover from the effects of PSI and amyloid load in the brain. Failure to recover or compensate for the effects of PSI on the LASSI-L distinguishes the LASSI-L from other widely used neuropsychological tests and appears to be sensitive to subtle cognitive impairments and increasing amyloid load.

- Curiel, RE, Crocco, E, Rosado, M., Duara, R., Greig, MT, Raffo, A and Loewenstein, DA. A Brief Computerized Paired Associate Test for the Detection of Mild Cognitive Impairment (MCI), in Community-dwelling Older Adults. Journal of Alzheimer's Disease. (2016) 6;54(2):793-9.

  ABSTRACT: MCI participants had lower scores on all indices relative to CN elders. A composite of two subscores correctly classified 85.3% of MCI and 84.4% of CN participants. Area under the ROC was higher relative to the MMSE, immediate memory for passages, and several subtests of a sensitive memory measure, the LASSI-L. The MITSI-L is a computerized test that can successfully differentiate MCI from CN participants. Area under the ROC curve exceeded that of global mental status and other memory measures. The effectiveness of the MITSI-L in detecting MCI, and its brief administration and portability render it worthy of further research.

- Loewenstein, DA., Curiel, RE., Buschke, H and Duara, R (In Press). Novel Cognitive Paradigms for the Detection of Memory Impairment in Preclinical Alzheimer's disease. Assessment.

  ABSTRACT: We describe the development of novel tests that are more cognitively challenging, minimize variability in learning strategies, enhance initial acquisition and retrieval using cues, and exploit vulnerabilities in persons with incipient AD such as the susceptibility to proactive interference (PSI), and failure to recover from PSI. The advantages of various novel memory assessment paradigms are examined as well as how they compare to traditional neuropsychological assessments of memory. Finally, future directions for the development of more effective assessment paradigms are suggested.

Patents: None at the time of reporting

3. **Grant #5AZ03:** Florida Consortium For African-American Alzheimer's Disease Studies (FCA3DS)

   Principal Investigator: Nilufer Ertekin-Taner, PhD

   Organization: Mayo Clinic Florida

   Progress Report: The FCA^{3}DS database, a relational Microsoft SQL database, has been designed to collect diagnosis and demographics information. An entity relationship diagram and lookup tables were created and populated with data to enforce data integrity. Institutional Review Board (IRB) approval was obtained at the University of South Florida and recruitment efforts were completed,

   Sample collection protocols for whole blood and PaxGene ribonucleic acid (RNA) were generated and disseminated to all four sites. Whole exome sequencing (WES) was completed and analyzed for 250 African-American subjects; 137 with Alzheimer's disease (AD) and 113 in the control group. Databasing of all samples and subjects, in addition to Data Quality Control (QC) were completed. AD risk associating variants were identified in known early-onset AD (EOAD), late-onset AD (LOAD) and novel genes.

   Follow On Funding:

   - Mayo Clinic Office of Health Disparities Research Pilot Grant $35,000
   - National Institutes of Health R03 Grant $ 50,000

   Collaborations: None at the time of reporting

   Journals:

   - "Comprehensive screening for disease risk variants in early-onset AD genes in African-Americans identifies novel PSEN variants." Authors: Aurelie N'Songo[1], Minerva M. Carrasquillo[1], Xue Wang[2], Thuy Nguyen[1], Yan Asmann[2], Steven G. Younkin[1], Mariet Allen[1], Ranjan Duara[3], Maria T. Greig Custo[3], Neill Graff-Radford[4], Nilüfer Ertekin-Taner[1],4#. Authors Institutions: 1. Mayo Clinic, Department of Neuroscience, Jacksonville, FL, USA 32224. 2. Mayo Clinic, Department of Health Science Research, Jacksonville, FL, USA 32224. 3. Wien Center for Alzheimer's Disease and Memory Disorders, Mount Sinai Medical Center, Miami Beach, FL 33140. 4. Mayo Clinic, Department of Neurology, Jacksonville, FL, USA 32224.

     ABSTRACT: We conducted a comprehensive screening of rare coding variants in an African-American cohort to identify novel pathogenic mutations within the early-onset Alzheimer's disease (EOAD) genes (APP, PSEN1 and PSEN2) in this understudied population. Whole-exome sequencing (WES) of 238 African-American subjects identified 6 rare missense variants within the EOAD genes, which were observed in AD cases but never among controls. These variants were analyzed in an independent cohort of 300 African-American subjects in which PSEN2:NM_000447:exon5:c.T331C:p.Phe111Leu and PSEN1-minilin rs777923890 variants were again not observed, indicating that these novel rare variants, may contribute to AD risk in this population. (In press, Journal of Alzheimer's Disease).

- "African-American exome sequencing identifies potential risk variants at Alzheimer's disease loci." Authors: Aurelie N'Songo1, Minerva M. Carrasquillo, PhD[1], Xue Wang, PhD[2], Jeremy D Burgess[1], Thuy Nguyen[1], Yan W. Asmann, PhD[2], Daniel J. Serie[2], Steven G. Younkin, MD, PhD[1], Mariet Allen, PhD[1], Otto Pedraza, PhD[3], Ranjan Duara, MD[4], Maria T. Greig Custo, MD[4], Neill Graff-Radford, MD[5], Nilüfer Ertekin-Taner, MD, PhD[1],5#. Authors Institutions: 1. Mayo Clinic, Department of Neuroscience, Jacksonville, FL, USA 32224. 2. Mayo Clinic, Department of Health Science Research, Jacksonville, FL, USA 32224. 3. Mayo Clinic, Department of Psychiatry and Psychology, Jacksonville, FL, USA 32224. 4. Wien Center for Alzheimer's Disease and Memory Disorders, Mount Sinai Medical Center, Miami Beach, FL 33140. 5. Mayo Clinic, Department of Neurology, Jacksonville, FL, USA 32224.

  ABSTRACT: In African-Americans, we sought to systematically identify coding Alzheimer's disease (AD) risk variants at the previously reported AD genome-wide association study (GWAS) loci genes. We identified coding variants within genes at the 20 published AD GWAS loci by whole exome sequencing of 238 African-American participants; validated these in 300 additional participants; and tested their association with AD-risk in the combined cohort of 538 and with memory endophenotypes in 319 participants. Two ABCA7 missense variants (rs3764647 and rs3752239) demonstrated significant association with AD-risk. Variants in MS4A6A, PTK2B and ZCWPW1 showed significant gene-based association. Additionally, coding variants in ZCWPW1 (rs6465770) and NME8 (rs10250905 and rs62001869) showed association with memory endophenotypes. Conclusions: Our findings support a role for ABCA7 missense variants in conferring AD-risk in African-Americans, highlights allelic heterogeneity at this locus, suggests presence of AD risk variants in MS4A6A, PTK2B and ZCWPW1, nominates additional variants that may modulate cognition and importantly provides a thorough screen of coding variants at AD GWAS loci that can guide future studies in this population. (Under 2nd review, Neurology Genetics).

Patents: None at the time of reporting

4. **Grant #5AZ04:** Developing Biotherapies For Alzheimer's Disease

   Principal Investigator: Jada Lewis, PhD

   Organization: University of Florida

   Progress Report: Cohorts were injected with tau transgenic and non-transgenic mice with Interleukin 10 (IL-10) and muscarinic acetylcholine receptor subtypes 3 (M3) to determine if they could modulate tauopathy. Six new adeno-associated viral vectors (AAVs) were produced to test for their ability to modulate tauopathy in subsequent periods. Also, 86 candidate gene fly lines were obtained to assess their ability to modulate toxicity observed in frontotemporal dementia (FTD). Two additional AAV-modifiers were tested and it was determined that they needed to be remade. Multiple genes were identified that likely serve a protective role against FTD in the fly model.

Mice were collected from all the AAV-modifier cohorts and found that M3 and IL-10 both significantly influence brain weight (tau-associated neurodegeneration). Evaluating tau pathology and biochemical changes is still in process, though initial results are promising. It was found that glial cell-derived neurotrophic factor (GDNF) AAV is not well tolerated in the mice and impair the behavioral functioning of even non-transgenic mice. Twenty-one suppressors of the TDP-43 gene and 6 suppressors of the C9ORF72 gene were identified and linked to FTD in the fly.

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 #5AZ05:** Therapeutic Potential Of PGE2 EP1 Receptor Selective Antagonist

Principal Investigator: Sylvain Doré, PhD

Organization: University of Florida

Progress Report: The project adapted surgical protocol and performed pilot experiments to minimize variability in the anatomical and behavioral outcomes in permanent distal middle cerebral artery occlusion (pdMCAO) stroke model. Optimized behavioral testing was completed to reproducibly detect behavioral changes following pdMCAO. Performed scheduling for all surgeries and experiments for the project, allowing appropriate aging of the experimental animals and timely execution of all surgical procedures, behavioral tests and analyses. Prepared formulation for injections of prostaglandin E 1 (EP1) antagonist and vehicle, aliquoted and coded for blinded treatment. Two strains of Alzheimer's disease (AD) mice were utilized and corresponding matching controls: 72 Tg2576 AD mice and 60 control mice (non-carrier), 11-18 weeks old, males; 72 APPswe/PS1 AD mice and 60 control mice, six to eight weeks old males.

All surgical procedures, tests, and analyses were randomized and performed in a blinded fashion. Experiments were conducted to test the efficacy of the selective antagonists, ONO-8713, compared to vehicle in Tg2576 mice and wild type control subjects to pdMCAO and sham surgeries. The surgical procedures, treatments and behavioral tests were performed in 82 mice.

All drug treatments, behavioral training, tests and stroke surgeries were completed for the three cohorts. All brains were extracted and sliced. Statistical analyses was completed and will be part of abstracts and papers that will be submitted 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

6. **Grant #5AZ06:** Modulation Of Arginine Metabolism And Polyamines To Mitigate Alzheimer's Disease Pathology

   Principal Investigator: Carlos T. Moraes, PhD

   Organization: University of Miami

   Progress Report: The cause of Alzheimer's Disease (AD) is unknown; however, there is evidence that defects in energy production by mitochondria have a major role in neuronal loss. This hypothesis was explored by using genetically modified mice produced and characterized in our lab to provide new leads for therapeutic interventions in Alzheimer's disease.

   Although the main topic of this lab is mitochondrial dysfunction, because of the Ed and Ethel Moore Alzheimer Research Program grant we established an Alzheimer's disease program. A project trainee, Dr. Milena Pinto, was promoted to assistant professor to work exclusively in neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease. With Dr. Pinto as the principal investigator, we have applied for a recent Florida biomedical grant on Alzheimer's disease that was not funded. However, she has partnered with Bernard S. Baumel, MD, an Alzheimer's disease-specialized physician in our Department of Neurology, to study the therapeutic approach of stem cells for Alzheimer's disease.

   Follow On Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals: Pioglitazone ameliorates the phenotype of a novel Parkinson's disease mouse model by reducing neuroinflammation. Milena Pinto, Nadee Nissanka, Susana Peralta, Roberta Brambilla, Francisca Diaz and Carlos T. Moraes. Molecular Neurodegeneration 11:25 (2016).

   Patents: None at the time of reporting

7. **Grant #5AZ07:** Targeting The Slingshot-Cofilin Pathway In Alzheimer's Disease

   Principal Investigator: David E. Kang, PhD

   Organization: University of South Florida

   Progress Report: The aim of this research was to perform in vitro and in silico screening for Slingshot inhibitors. Utilized were the secure digital (SD) and SMILES files for the Chembridge Central Nervous System-Set library (>50,000 compounds) to virtually dock chemical structures to the known crystal structure of slingshot (SSH2 and SSH1) catalytic sites. Based on computational models of favorable binding interactions with Slingshot, the top 2000 compounds were selected. Of these compounds, 667

compounds that were available in the one micro-mol quantity were purchased. Initial physical screening using the p-nitrophenyl phosphate (pNPP) phosphatase assay from the first ~100 compounds, identified seven hits. Of these, five were slingshot inhibitors and two were allosteric modulators. Follow up experiments demonstrated one inhibitor compound with a half maximal inhibitory concentration (IC50) in the nanomolar range, whereas other inhibitors had IC50 values in the low micromolar range. Preliminary cell-based assays also showed that the inhibitors increased Cofilin phosphorylation as expected.

The physical screening was completed of the remainder of the 667 compounds. Three additional Slingshot inhibitors and two allosteric activators were identified by in vitro pNPP assay. Some issues were initially encountered with Slingshot protein purification, which was later resolved. As such, the project staff were unable to confirm the activity of the new batch of slingshot inhibitors and activators by cell-based assays.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals:

- Woo, J. A., Zhao, X., Khan, H., Penn, C., Joly-Amado, A., Wang, X., Weeber, E., Morgan, D., and Kang, D. E. (2015). Slinghot-Cofilin activation mediates mitochondrial and synaptic dysfunction via Abeta ligation to beta 1-integrin conformers. *Cell Death & Differentiation*. Doi: 10.1038/cdd.2015.5.

Patents: None at the time of reporting

8. **Grant #5AZ08:** ApoE And Gender Effects On Alzheimer's Disease And Cerebral Amyloid Angiopathy

   Principal Investigator: Guojun Bu, PhD

   Organization: Mayo Clinic of Florida

   Progress Report: APOE4 is a strong genetic risk factor for Alzheimer's disease (AD) and cerebral amyloid angiopathy (CAA). Sex-dependent differences have been shown in AD, as well as, cerebrovascular diseases. Thus, research staff examined the effects of Apolipoprotein E 4 (APOE4), sex, and pathological components on CAA in AD subjects. Among 428 autopsied brain samples from pathologically confirmed AD available in Mayo Clinic Brain Bank, the cases with severe CAA (n=60) or without CAA (n=39) were selected for bio chemical analysis of CAA-related molecules including amyloid-B (AB) and apolipoprotiein E (apoE) in the temporal cortex by ELISA. Amyloid beta 40 (AB40) levels were higher in the temporal cortex from AD cases with CAA than those without CAA, where it was significant in female APOE4 carriers when stratified by APOE4 status and sex. APOE4 was associated with decreased soluble apoE and increased insoluble apoE, independent of CAA status and sex. These results indicate that APOE genotype and sex differentially modify the risk and severity for CAA in AD. A manuscript is being developed summarizing these findings for publication.

In addition, most of the brain samples were also subjects to systems-based analyses including ribonucleic acid (RNA) sequencing (n=75), lipidomics (n=99) and genome-wide association study (GWAS) (n=94) with a goal to identify genes and pathways that underlie the effects of sex and APOE4 on CAA. The data collection and analysis are currently ongoing, which will be completed within the near future.

Follow On Funding: National Institute on Aging $5,327,310

Collaborations: None at the time of reporting

Journals: Impact of sex and APOE4 on cerebral amyloid angiopathy in Alzheimer's disease. Shinohara M, Murray ME, Frank RD, Shinohara M, DeTure M, Yamazaki Y, Tachibana M, Atagi Y, Davis MD, Liu CC, Zhao N, Painter MM, Petersen RC, Fryer JD, Crook JE, Dickson DW, Bu G, Kanekiyo T.Acta Neuropathol. 2016 Aug;132(2):225-34. doi: 10.1007/s00401-016-1580-y.
PMID: 27179972

Patents: None at the time of reporting

9. **Grant #5AZ09:** Epigenetic Approach For The Treatment Of Alzheimer's Disease

Principal Investigator: Claes Wahlestedt, MD, PhD

Organization: University of Miami

Progress Report: This grant allowed for significant concept development using an epigenetic small molecule to treat Alzheimer's disease (AD). This research demonstrated that targeting specific histone deacetylases to target several biomarkers of interest in AD was a feasible approach. Identification of various mechanisms that are likely to be involved in AD pathogenesis were elucidated, in addition, to the ability to hone in on drugs that regulate key components of this disease.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: Volmar C-H, Salah-Uddin H, Halley P, Lambert G, Mehta N, Wodrich A, Dorcius D, Wahlestedt C (2015) An Epigenetic Approach for the Modulation of Amyloid Precursor Protein (APP) Processing and Improvement of Memory in Alzheimer's Disease. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol 40:S470.

Volmar C-H, Salah-Uddin H, Halley P, Lambert G, Wodrich A, Manoah S, Patel N, Mehta N, Sartor GC, Janczura K, Desse S, Dorcius D, Wahlestedt C (2016) Epigenetic modulation of amyloid precursor protein (APP) metabolites and other Alzheimer's disease biomarkers In vitro and In vivo. In: 2016 Neuroscience Meeting Planner, Program No. 785.09 G33. San Diego, CA: Society for Neuroscience. Online.

Patents: None at the time of reporting

10. **Grant #5AZ10:** Flavonoid-Diosmin, A Novel Gamma-Secretase Modulator, For The Treatment Of Alzheimer's Disease

    Principal Investigator: Jun Tan, MD, PhD

    Organization: University of South Florida

    Progress Report: Diosmin reduces amyloid beta (AB) deposits and tau phosphorylation, as well as, total soluble and insoluble tau with minimum doses. Data generated from the measurement of amyloid precursor protein (APP) processing as examined by the analysis of Soluble b-amyloid Precursor Protein Alpha (sAPPa B) and carboxy terminal fragments (CTFs) are informative as to the actions of diosmin/diometin for inhibiting y-secretase cleavage of amyloid precursor protein (APP). Importantly, this study provides knowledge regarding diosmin's therapeutic effect with minimum doses to reduce Alzheimer's disease (AD)-like pathology. Such data are suggestive of dietary modifications and/or supplementation which would be applied to the human condition. Since the normal aging of the human brain is characterized by an increase in newly synthesized oligomers of amyloid beta (AB) and expression of pro-inflammatory cytokines along with a decrease of anti-inflammatory cytokines, such as Interleukin (IL-10 and IL-4), as expected the microglial cells isolated from older 3XTg-AD mice are more easily skewed towards a muscarinic 1 (M1)-like phenotype than those isolated from young mice. However, microglia isolated from diosmin-fed 3XTg-AD mice strongly preserves an muscarinic 2 (M2) phenotype based on this lab's previous studies and preliminary results. This is also supported by the fact that diosmin's metabolite, diosmetin, has other beneficial actions such as an antioxidant effect, which may act to override potentially harmful effects in microglia elicited by y-secretase inhibition, such as mitochondrial dysfunction and subsequent promotion of amyloidogenic, an APP processing.

    A preserved M2 phenotype in microglia derived from diosmin-fed 3XTg-AD mice were evidenced by M2 phenotype markers Peroxisome Proliferator-Activated Receptor (PPARy) activation, increased microglial phagocytosis, decreased pro-inflammatory intracellular signaling mitogen-activated protein kinase (MAPK), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and resultant decreased M1 cytokine profile. Diosmin/diosmetin decreases T Helper (Th1/Th2) cytokine and M1/M2 microglial ratios in primary microglial cultures and strongly enhance M2 phenotype polarization in the present of IL-4 and dampen or abolish the M1 response to tetrahydrofurfuryl alcohol (THFa). IL-4 decreases Notch1 expression in microglial cells since an up-regulation of Notch1 is associated with pro-inflammatory events. These data are important to begin to understand how diosmin/diosmetin mechanistically works on the cellular level to reduce AD pathology and cognitive impairment.

    Follow On Funding: National Institute on Aging $275,000

    Collaborations: None at the time of reporting

    Journals: None at the time of reporting

    Patents: US 8802638 B1 entitled "Flavonoid treatment of glycogen synthase kinase-based disease"

ABSTRACT: The flavonoid luteolin reduces amyloid-β peptide (Aβ) generation. Luteolin is also a selective GSK-3 inhibitor that 1) decreases amyloidogenic γ-secretase APP processing, and 2) promotes presenilin 1 (PS1) carboxyl-terminal fragment (CTF) phosphorylation. GSK-3α activity is essential for both PS1 CTF phosphorylation states and PS1-APP interaction. These findings were validated in vivo, using a Tg2576 Alzheimer's disease model system. Luteolin treatment decreased soluble Aβ levels, reduced GSK-3 activity, and disrupted PS1-APP association. In addition, Tg2576 mice treated with diosmin, a glycoside of a flavone structurally and functionally similar to luteolin (diosmetin), displayed significantly reduced Aβ pathology as well.

11. **Grant #5AZ11:** Modulation Of Arginine Metabolism And Polyamines To Mitigate Alzheimer's Disease Pathology

Principal Investigator: Daniel C. Lee, PhD

Organization: University of South Florida

Progress Report: Overall, data indicate a mixed beneficial and worsening of affects depending on the task and treatment. Tau loads in each mouse were used to correlate behavioral measures. Importantly, arginase 1 (Arg1), arginine decarboxylase (ADC) and arginine deiminase (ADI) increased motor learning and performance compared to a control group empty capsid. This may be an important finding regarding tauopathies that impact motor deficits.

Histopathology on various groups were collected. Behavioral measures confirm improved motor deficits with all three treatments, thus polyamines improve tau induced motor impairment. An improvement on cognition was not observed; however, there was no worsening from treatments. In a parallel experiment, it was found that arginine deiminase (ADI) significantly reduced the tau tangles and phosphor tau. A pursuit of arginine decarboxylase (ADC) and its effects on tau pathology was initiated.

Unfortunately, tau transgenic mice did not produce the robust behavioral defects compared to non-transgenic mice reported in the literature, although, these mice showed copious amounts of tau with pathology. This makes the data difficult to interpret. Several significant effects with, both, the arginine deiminase (ADI) treatment and with arginine decarboxylase (ADC) treatment were found. Although both deplete arginine, each modifies behavior differently. ADI significantly increases motor activity in non-transgenic mice, however, ADI appears to impair spatial working memory in non-transgenic mice. Additionally, ADI impair fear-associated memory consolidation and/or recall in non-transgenic mice. An important finding suggests that ADI increased motor learning and motor memory in tau transgenic mice. Thus, the PS19 (tauP301S) model did not produce the consistent behavioral defects such as cognitive impairment as previously reported, thus it cannot be concluded that the treatment impacted cognitive deficits. ADI showed no effect on total tau levels, whereas ADC significantly decreased total tau levels.

Follow On Funding: Bright Focus Foundation $250,000 for 2015-2018 and Alzheimer's Association $150,000

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents:

- USF Ref No.: 15A023 Identification of Arginine Deiminase Gene Therapy for Disordered Proteins: February 26, 2015 U.S. *Patent pending*

- USF Ref. No.: 16B142 - Exploiting Allosteric Antagonists to GPRC6a to Mitigate Proteinopathies November 1, 2016 *Patent pending*

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