Alzheimer's Disease Research Grant Advisory Board

Ed and Ethel Moore Alzheimer's Disease Research Program

2017-2018 Report

Rick Scott
Governor

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

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

Alzheimer's disease is a debilitating brain disease that affects approximately 5.4 million Americans, including 520,000 Floridians, over the age of 65.[1] It is estimated that by 2025, over 720,000 seniors will be living with this disabling disease in the state of Florida.[1] Alzheimer's disease is the sixth leading cause of death in Florida[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 administered 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.

This fiscal year, the legislature provided $5 million to fund new research grant projects within the state. Appendix A details all newly awarded grants and Appendix B details all active grants in fiscal year 2017-2018. Information regarding progress reports, follow on funding, publications and patents of each active grant is also found in Appendix B.

Annually, the Alzheimer's Disease Research Grant Advisory Board (Advisory Board) submits a fiscal year progress report, as required by section 381.82, Florida Statutes. The report must include:

a) For each research project supported by grants or fellowships awarded under the program:

1. A summary of the research project and results or expected results of the research.

2. The status of the research project, including whether it has concluded or the estimated date of completion.

3. The amount of the grant or fellowship awarded and the estimated or actual cost of the research project.

4. A list of principal investigators under the research project.

5. The title, citation, and summary of findings of a publication in a peer-reviewed journal resulting from the research.

6. The source and amount of any federal, state, or local government grants or donations or private grants or donations generated as a result of the research project.

7. The status of a patent, if any, generated from the research project and an economic analysis of the impact of the resulting patent.

8. A list of postsecondary educational institutions involved in the research project, a description of each postsecondary educational institution's involvement in the research project, and the number of students receiving training or performing research under the research project.

b) The state ranking and total amount of Alzheimer's disease research funding currently flowing into the state from the National Institutes of Health.

c) Progress toward programmatic goals, particularly in the prevention, diagnosis, treatment, and cure of Alzheimer's disease.

d) Recommendations to further the mission of the program.

ALZHEIMER'S DISEASE RESEARCH GRANT ADVISORY BOARD
OVERVIEW AND MEMBERSHIP

The Advisory Board created 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 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.

Alzheimer's Disease Research Grant Advisory Board members as of December 12, 2017 (Biographical Statements or Curriculum Vitae are 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

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

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

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

Between fiscal years 2012-2014, Florida received $12,017,087 from the National Institutes of Health (NIH) to perform Alzheimer's disease research, ranking 12th, 13th and 11th in national federal funding, respectively, per the NIH's National Center for Health Statistics. By fiscal year 2015, NIH funding in the state of Florida nearly doubled to $22,729,691 and has doubled again, in fiscal year 2016, to $41,942,429 (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 $19,212,738 in the 2016 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 related 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 Wisconsin and Michigan, and saw the highest funding gains of the Top 10 ranked states in 2016, nearly double the rate of the 2nd ranked state, New York** (Figure 2 and 3).

Figure 1: National Institutes of Health Alzheimer's Disease Research State Funding and Rankings Fiscal Year 2016

State	Total Funding	Rank
CA	$164,358,757.00	1
NY	$116,998,284.00	2
MA	$99,254,887.00	3
PA	$75,637,872.00	4
IL	$54,114,221.00	5
MD	$46,134,689.00	6
FL	**$41,942,429.00**	**7**
MO	$35,913,339.00	8
TX	$33,728,735.00	9
MN	$28,424,194.00	10
NC	$22,865,222.00	11
WA	$20,672,022.00	12
WI	$19,178,081.00	13
MI	$17,466,791.00	14
GA	$15,987,859.00	15
OH	$15,047,467.00	16
IN	$14,197,398.00	17
AZ	$13,103,040.00	18
CT	$12,305,026.00	19
KY	$10,349,108.00	20

Fig.1 NIH Research Funding from the 2016 Fiscal Year Reporting Period: The top twenty ranked states in NIH funding for Alzheimer's disease is displayed. With over $41.9 million in NIH funding, Florida is ranked 7th in the nation. *Source: National Institutes of Health 2017, https://report.nih.gov/categorical_spending.aspx*
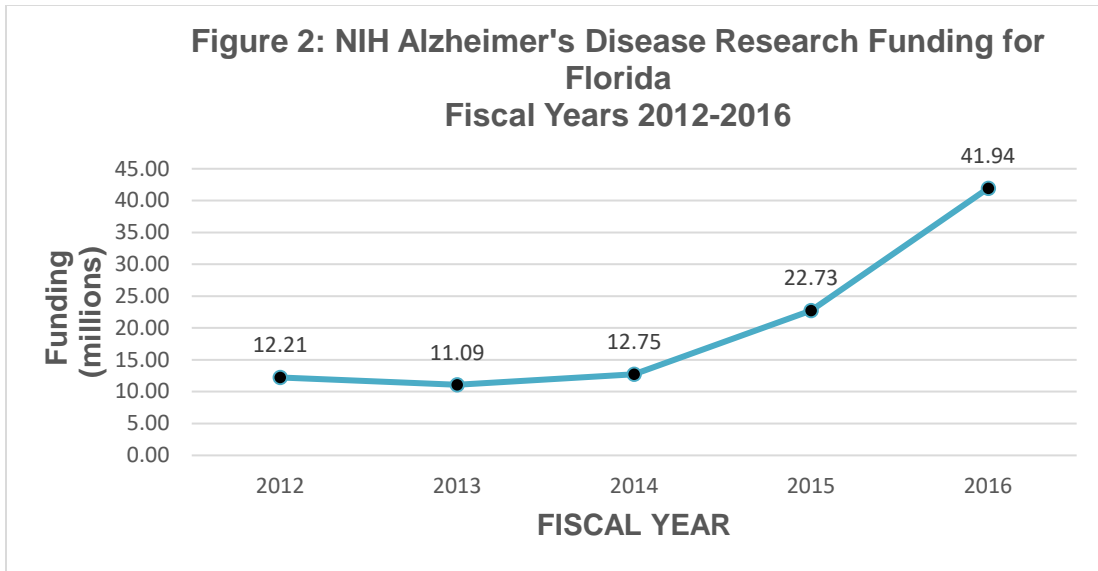
Figure 2: NIH Alzheimer's Disease Research Funding for Florida Fiscal Years 2012-2016

Fig. 2 NIH Research Funding Trends in Florida Fiscal Year 2012-2016: 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 and 2016 fiscal years each saw nearly a doubling of funding, leading to a 229% increase of funding since 2014. *Source: National Institutes of Health 2017, https://report.nih.gov/categorical_spending.aspx*
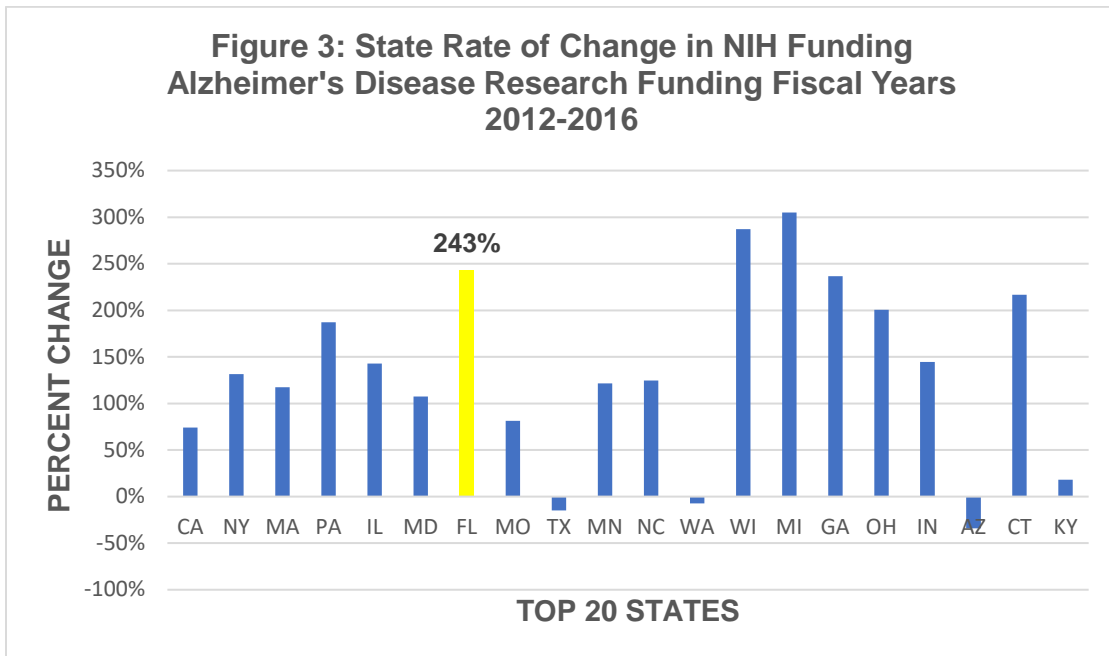

Figure 3: State Rate of Change in NIH Funding Alzheimer's Disease Research Funding Fiscal Years 2012-2016

Fig. 3 Change in NIH Research Funding in the Top 20 States Fiscal Years 2012-2016: This graph displays the rate of change in federal Alzheimer's disease research funding for the Top 20 states for fiscal years 2012-2016. Among the Top 10 ranked states in NIH funding for Alzheimer's disease, Florida saw the third highest funding gains since 2012 (243%). Between 2015 and 2016, Florida's NIH funding for Alzheimer's disease grew by 84.5% and has remained seventh for total funding.

Source: National Institutes of Health 2017, https://report.nih.gov/categorical_spending.aspx

PROGRESS TOWARD PROGRAMMATIC GOALS

The Advisory Board created a research agenda that 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

An Alzheimer's Disease Research and Awareness Symposium is planned for June 7 – 8, 2018 to showcase the advances identified by Florida researchers receiving Ed and Ethel Moore Alzheimer's Disease Research Program grant funds. The Department applied for a grant available through the Alzheimer's Association and was awarded funds to support the associated costs of holding this statewide event. The grants were offered by the Alzheimer's Association in collaboration with the Association of State and Territorial Health Officials to promote the Healthy Brain Initiative. The symposium is a partnership between the Alzheimer's Association and Florida Department of Elder Affairs to provide current information on the disease, the latest research findings from Florida researchers, and clinical trial opportunities. This symposium will increase collaboration and information sharing among research investigators and may result in new consortium grants.

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 Program with allocated funding to award $5,000,000 to 31 outstanding research projects from 12 different research organizations in Florida. Without this support, the eminent scientific advancements and discoveries in Alzheimer's disease would not be possible. Although great progress has been made, the Advisory Board requests that up to five percent of the funds allocated to this program be made available for costs associated with the administration of this program. These administrative expenses are consistent with the William G. Bankhead, Jr. and David Coley Cancer Research Program, as well as the James and Esther King Biomedical Research Program administered by the Department.

Currently, there are 73 active research grants managed by temporary staff at the Department. The expertise of permanent staff are needed for administering the statutory requirements of this research program. Temporary staff often have other duties and functions that compete for time

and effort. An online grant intake system is necessary for efficient management of research grant reporting, invoicing, and monitoring.

Additionally, these funds will be used to pay for a vendor to coordinate rigorous, scientific peer review of all grant applications. There is a great need for this expertise, especially in basic research science. Peer review scoring and comments on the quality of a grant application assists in making funding recommendations to the State Surgeon General. The peer review process involves many steps. In keeping with the peer review standards by the National Institutes of Health, the review should involve rigorous scientific critique of each research application by a panel. The Department has not been able to achieve these standards. Advisory Board members provide the peer review of the grant applications, whenever their expertise is an appropriate fit for the content and science of the research application. Each careful review and reviewer write-up of the critique may take one or more hours. After the grant application process is concluded, peer review comments are sent back to each grant applicant for further refinement of the grant application in the following year. Each year it has been more difficult to identify reviewers who are willing to volunteer their time to review our applications due to their own academic, clinical and research demands. This year, a total of 221 potential peer reviewers were contacted to yield 33 volunteer peer reviewers.

This statutory change of allowing up to five percent of the allocated funds for administrative functions will provide for reimbursement of travel expenses resulting from Advisory Board in-person meetings. In-person meetings allow for effective strategic planning and facilitate in-depth communication about critical research issues. Implementing these recommendations will ensure continued forward progress for this research program.

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 2017-2018 NEWLY AWARDED GRANTS (effective February 6, 2018)

Grant #	Organization	PI Name	Project Title	Award Amount
8AZ01	Ave Maria University	Barbosa, Antonio	Inhibiting Alzheimer's Disease By Modulating A Key Player In Plaque And Tangle Formation, SIRT1, By Regulating The Formation Of Nicotinamide Metabolites	$ 100,000
8AZ02	Florida Atlantic University	Modi, Jigar	Neuroprotection Of GCSF Gene therapy In Alzheimer's Disease	$ 100,000
8AZ03	Florida Institute of Technology	Liao, Yi	CO Releasing Polymer Nanoparticles For Treatment Of Alzheimer's Disease	$ 100,000
8AZ04	Florida International University	Nair, Madhavan	Therapeutic Role Of Withaferin A And CRID3 In The Prevention Of AD: A Novel Nanotechnology Approach.	$ 224,643
8AZ05	Florida State University	Carretta, Henry	Disparities In Health Services Utilization Across Racial/Ethnic Groups Among Persons With Alzheimer's Disease And Related Conditions	$ 100,000
8AZ06	Mayo Clinic Jacksonville	Murray, Melissa Erin	Quantitative Neuropathology And Biochemistry Of Survival Differences In Hispanic Americans With Alzheimer's Disease	$ 221,000
8AZ07	Mayo Clinic Jacksonville	Liu, Chia-Chen	Impact Of TREM2 Variants On Microglial Function And Alzheimer's Disease Pathology	$ 221,000
8AZ08	Mayo Clinic Jacksonville	Lucas, John A.	Evaluating The Impact Of A Dementia-Caring Community Model On African Americans With Alzheimer's Disease And Their Care Partners.	$ 200,000
8AZ09	Mayo Clinic Jacksonville	Melrose, Heather	Targeting Lrrk2 Activity To Modulate Tau Pathology	$ 99,999
8AZ10	Mayo Clinic Jacksonville	Ebbert, Mark T. W.	Identifying Drug Targets Using Long-Read Sequencing In Alzheimer's Disease And Control Brain Tissue	$ 100,000
8AZ11	Mount Sinai Medical Center	Goenaga, Sindy	Impact Of The Modified MindSet Training Program On Maintaining Optimal Function Among Early Alzheimer's Patients And Their Care Partners	$ 96,643
8AZ12	University of Central Florida	Teter, Ken	Protein Disulfide Isomerase Uses Conditional Disorder As A Disaggregase Mechanism To Detoxify Amyloid Beta Fibrils	$ 200,000
8AZ13	University of Central Florida	Hernandez, Florencio	Optical Characterization Of The Aggregation (Change In Size, Fibril Formation), Accompanying Structural Changes, And Membrane Pore Formation.	$ 200,000
8AZ14	University of Central Florida	Simmons, Elzbieta Sikorska	Factors Influencing Family Caregivers' Medical Decision-Making For Patients With Advanced Alzheimer's Disease	$ 95,784
8AZ15	University of Florida	Kesavalu, Lakshmyya	Periodontal Bacteria Augment Progression Of Abeta; And Tau Pathology	$ 221,000
8AZ16	University of Florida	Chakrabarty, Paramita	Towards Understanding The Biological Role Of Newly Discovered Alzheimer's Disease Susceptibility Genes Affecting Immune Function	$ 221,000
8AZ17	University of Florida	Cottler, Linda B.	Precision Public Health Approaches To Reduce Disparities In Memory Disorder Screening In Rural Minority Communities	$ 200,000
8AZ18	University of Florida	Yachnis, Anthony T.	Investigations Of Neuropathologies Targeted By Clinical Trials In Alzheimer's Disease Patients	$ 99,987
8AZ19	University of Florida	Streit, Wolfgang J.	Role Of Microglia In Primary Age Related Tauopathy And In Sporadic (Late-Onset) Alzheimer's Disease	$ 96,643
8AZ20	University of Florida	Xu, Guilian	Seeded Interactions Of Abeta; And Neurofibrillary Tangle Pathologies In Mouse Models	$ 99,577

8AZ21	University of Miami	Curiel, Rosie	Postdoctoral Fellowship In Neuropsychology	$ 89,304
8AZ22	University of Miami	Alperin, Noam	Cardiovascular And Lifestyle Stressors Of Hippocampus And AD Related Brain Regions	$ 221,000
8AZ23	University of Miami	Loewenstein, David	The Relationships Between Multimodal Neuroimaging Biomarkers And A Novel Cognitive Stress Test (CST) Among Ethnically Diverse Older Adults	$ 450,844
8AZ24	University of Miami	Toborek, Michal	Extracellular Vesicles As Novel Therapeutic Targets In Alzheimer's Disease	$ 221,000
8AZ25	University of Miami	Griswold, Anthony	Identification Of Noncoding Functional Variant(s) Underlying Alzheimer Disease GWAS Hits	$ 100,000
8AZ26	University of Miami	Dykxhoorn, Derek	Investigating The Role Of SORL1 In Alzheimer's Disease	$ 200,000
8AZ27	University of South Florida	Selenica, Maj-Linda B.	Emerging Role Of Tau Citrullination In Alzheimer's Disease	$ 100,000
8AZ28	University of South Florida	Nash, Kevin	Microglial Phenotype In Alzheimer's Disease.	$ 100,000
8AZ29	University of South Florida	Kang, David E.	Divergent RanBP9 Signaling In Tau Pathogenesis	$ 221,000
8AZ30	University of South Florida	Lee, Daniel C.	Exploiting GPRC6a Antagonists To Mitigate Tau Deposition	$ 200,000
8AZ31	Relinquished		Received NIH funding for this grant project	
8AZ32	The Roskamp Institute, Inc.	Keegan, Andrew	Longitudinal assessment of BDNF levels with Bacopa monnieri treatment in those at risk of developing Alzheimer's dementia	$99,576

1. **Grant #8AZ01:** Inhibiting Alzheimer's Disease By Modulating A Key Player In Plaque And Tangle Formation, SIRT1, By Regulating The Formation Of Nicotinamide Metabolites

   Principal Investigator: Antonio Barbosa, PhD

   Organization: Ave Maria University

   Abstract of Proposed Research: Increasing evidence suggests that Alzheimer's disease (AD) is linked to changes in the metabolic profiles of patients. Accordingly, specific metabolites may work to promote neuron survival. Therefore, this study will investigate how the metabolite methyl-nicotinamide (Me-NAM) acts on the key metabolic protein sirtuin 1 (SIRT1) to promote neuron survival. To accomplish this, the institution has established a multi-investigator team of researchers in biochemistry, medicinal chemistry, and biology to address the major grant priority area of novel therapeutic targets and strategies. SIRT1 is a deacetylase protein that prevents the formation of Tau tangles and amyloid beta plaque build- up in AD. Increasing SIRT1 activity has been shown to reduce these Tau tangles and amyloid beta plaque build-up in mouse models of AD. One metabolite byproduct of SIRT1 activation is nicotinamide, a component of the vitamin B3 complex. Nicotinamide is converted to methyl-nicotinamide (Me-NAM) by the Nicotinamide N-Methyltransferase (NNMT) protein in the cells. Me-NAM was once considered an inactive metabolite, but it was recently found to stabilize SIRT1 protein in model liver cells. The research team hypothesize that increased NNMT activity will increase Me-NAM levels and thereby directly enhance beneficial SIRT1 protein stability and activity in neurons. Their multi-investigator team of researchers will study this hypothesis with three goals. First, the researcher team will investigate how Me-NAM stabilizes SIRT1, a mechanism that is currently not known. Second, they will explore a detection system for Me-NAM metabolites and NNMT activity. Finally, the team will perform a small drug screen to identify chemical compounds that modulate NNMT activity and determine the effect of this increased NNMT activity on SIRT1 stability. The research team will use known SIRT1 activating compounds synthesized by their students as positive controls for their studies. They anticipate that this research will be beneficial for the discovery of new therapies for AD.

2. **Grant #8AZ02:** Neuroprotection Of GCSF Gene Therapy In Alzheimer's Disease

   Principal Investigator: Jigar Modi, MD, PhD

   Organization: Florida Atlantic University

   Abstract of Proposed Research: Gene therapy offers unique opportunities for translational medicine for Alzheimer's disease (AD) by refining the products of defective genes in diseases and/or offering vital biologics from endogenous sources for tissue recovery processes. However, validating methods for the delivery, distribution and expression of the exogenous genes from such therapy can generally not be applicable to monitor effects over the long term because they are invasive. Most GCSF (Granulocyte Colony Stimulating Factor) gene stimulate production of GCSF protein, is a

glycoprotein that stimulates production of granulocytes and stem cells. The laboratory staff have recently noted that it has neuroprotective properties as well as facilitation of stem cell differentiation. In 2016, they reported that human granulocyte colony-stimulating factor (hG-CSF) cDNA incorporated in scAAV-type 2 adeno-associated virus, as introduced through eye drops at multiple time points after cerebral ischemia utilizing bilateral carotid occlusion for 60 min (BCAO-60) resulted in substantial drop in mortality rates, cerebral atrophy, and neurological deficits in C57black6 mice. In application to AD, GCSF gene treatment has been found to improve the spatial learning performance and reduce amyloid depositions in the hippocampus and entorhinal cortex of mice animal models, however the mechanism of this interaction is still unclear. Given that AD is the 6th leading cause of death in United States of America, understanding the neuroprotective and neurogenesis mechanism of GCSF gene as a potential therapeutic agent for neurodegenerative diseases such as Parkinson's disease and AD is highly desirable. In this new study addressing AD, the research team proposes that the GCSF gene treatment could serve as potential therapeutic agents for AD. They propose that GCSF gene could be a potential therapeutic agent for AD by first testing its effects in an in vitro setting as proof of concept. Preliminary results confirmed that cell survival rate of PC12 cells was significantly improved after exposure to amyloid-beta (A-Beta) toxicity when compared to baseline values ($P < 0.05$) with GCSF gene intervention. In examining G-CSF gene treatment of PC12 cells exposed to A-Beta toxicity, the team of researchers demonstrated an increased survival rate in GCSF gene treated cells when compared to control cells. In examining the therapeutic potential of GCSF gene in PC12 cells, the GCSF gene was found to be neuroprotective against either glutamate induced toxicity, hypoxia /re-oxygenation. GCSF gene treatment also showed dose dependency action against A-Beta over varying concentration of 20 pfu/cell and 40 pfu/cell. Overall, this is an indication that GCSF gene can in fact protect against A-Beta toxicity, a bio marker of AD. Their ongoing studies during this grant period will involve examining the neuroprotective potential of GCSF gene in vivo using rodent models of AD. They plan to further understand the mechanism and to determine effects of GCSF gene on cognitive functions using the animal model for AD.

3. **Grant #8AZ03:** CO Releasing Polymer Nanoparticles For Treatment Of Alzheimer's Disease

Principal Investigator: Yi Liao, PhD

Organization: Florida Institute of Technology

Abstract of Proposed Research: This pilot project is aiming to develop a nanomedicine for Alzheimer's disease (AD). More than 35 million people worldwide suffer from AD including about 5.5 million Americans. Currently, there is no cure for AD. Although carbon monoxide (CO) is known as a toxic gas, it is naturally produced in small quantities and plays important roles in biological functions. Studies in the past two decades have shown many beneficial effects of CO. In fact, inhaled CO has entered clinical trials for treatment of inflammation and cardiovascular disorders. It was found that level of CO increased in the brains of AD patients. A later study showed that Heme oxygenase-1 produced CO to protect brain cells from damage caused by amyloid related to AD, which is consistent with the neuroprotecting effects of CO suggested by many studies. Since CO is toxic at high level, control over the dose of CO is important. Carbon monoxide releasing molecules (CORMs) have been studied in the past decade

for controlled release of CO. Beneficial effects of a CORM on brain cells have been demonstrated by an in vitro study. However, there is no report showing that the CORM can pass blood-brain barrier. In this project, the research team will develop brain-delivery polymer nanoparticles loaded with CORMs, and study their CO releasing profile. These nanoparticles are expected to be able to release CO in the brains of AD patients, strengthen the self-protecting measures naturally adopted, and ease the symptoms of AD. The brain-delivery nanoparticle is based on polysorbate 80-coated polybutylcyanoacrylate nanoparticle, which has been widely used to deliver different drugs to brain. Two types of CORMs will be loaded to the nanoparticles. The first type of CORM releases CO through a hydrolysis mechanism. The second type releases CO upon photo-irradiation and thus can be selectively activated at the sites where amyloids are observed. Results of this pilot project will allow the therapeutic effects of CO on AD to be carefully studied and the related drugs to be developed in the future.

4. **Grant #8AZ04:** Therapeutic Role Of Withaferin A And CRID3 In The Prevention Of AD. A Novel Nanotechnology Approach

Principal Investigator: Madhavan Nair, PhD

Organization: Florida International University

Abstract of Proposed Research: Alzheimer's Disease (AD) is a growing threat to healthcare in the aging population and is marked by the accumulation of amyloid beta deposition in the brain and the pathology is enhanced by neuroinflammatory process. Inflammasomes are recently discovered multiprotein signaling complexes and are known to trigger inflammatory proteins such as IL-1beta; these inflammatory response proteins are known to play a significant role in the genesis of AD. Among the inflammasome complexes, a nucleotide-binding oligomerization domain-like receptor, NLRP3 and nuclear factor kB (NFkB) are the major neuroinflammatory pathways that lead to AD. Therefore, therapeutic drugs which can target both NFkB and NLRP3 activation will play a major role in reducing amyloid beta levels and prevention of neuropathology of AD. In the researchers' preliminary studies, they have reported for the first time that Withaferin A (WA), an extract from Withania somnifera plant, known to inhibit NFkB activation, improved synaptic plasticity and neuronal spine density; and significantly inhibited amyloid beta production and amyloid beta induced neurodegeneration. Furthermore, they have also demonstrated that cytokine release inhibitory drug 3 (CRID3), an inhibitor of NLRP3 significantly prevented neuroinflammation in their in vitro model system. Therefore, use of these therapeutic drugs targeting both NFkB and NLRP3 will have a translational significance in the prevention of neuroinflammation and associated neurodegeneration in AD patients. However, these drugs are impenetrable to the brain to prevent neuroinflammation and subsequent neurodegeneration. The use of nanotechnology in medicine has exciting prospects for the development of a novel drug delivery system to the brain across the Blood Brain Barrier (BBB). The researchers recently developed a manuscript and patented technology (US20130317279 A1 and WO patent: CT/US2013/068698) that describes magneto-electric nanoparticles (MENPs) as a novel drug carrier which offers unique capabilities including its low energy and dissipation-free on-demand drug release across BBB. Accordingly, the research team will use MENPs as carrier

molecules to deliver WA and CRID3 across BBB to inhibit the NFkB and NLRP3 mediated neurodegeneration in AD. To accomplish this goal, they plan on using their patented novel nanotechnology approach. Thus in Aim #1, they will investigate the effect of Withaferin A and CRID3 in inhibition of amyloid beta induced NFkB and NLRP3 activation and the associated down-stream inhibition of pro-inflammatory cytokines and amyloid beta production in mixed in-vitro microglia and SH-APP (APP over expressing SH- SY5Y neuronal cell line) cell culture models. In Aim #2, they will develop and characterize MENP bound WA and CRID3 within the liposomes (to increase the drug stability, bioavailability and target specific brain delivery of these drugs); and study its non-invasive BBB transmigration, on-demand controlled release and therapeutic efficacy of the developed cargo using the in-vitro BBB model. Lastly, in Aim #3 the researchers will study the therapeutic efficacy (synaptic plasticity and neurobehavioral) of this novel nanoformulation in APP/PS1 AD mouse model.

5. **Grant #8AZ05:** Disparities In Health Services Utilization Across Racial/Ethnic Groups Among Persons With Alzheimer's Disease And Related Conditions

Principal Investigator: Henry Carretta, PhD, MPH

Organization: Florida State University

Abstract of Proposed Research: Alzheimer's disease (AD) is the most common form of dementia. It is a progressive neurodevelopmental disease associated with high morbidity and mortality. Some research suggests that risk factors for heart disease and stroke, e.g. high blood pressure and high cholesterol, increase the risk of AD. Florida experiences an elevated population burden due to AD. Extant evidence suggests that there are significant disparities in prevalence, treatment and diagnosis rates, use of services, and mortality across racial and ethnic groups for AD and related disorders. The overall purpose of the project is to describe the prevalence, use of services, comorbid conditions and mortality across racial and ethnic groups with AD and related disorders and their relationship with known comorbid risk factors to provide fundamental insights for potentially modifiable risk factors. The project will help to define and quantify the nature and size of racial/ethnic differences and disparities in service utilization and access to care as the first step is establishing a blueprint for a resolution. The Florida State research team proposes the following research questions (RQ) to more precisely parse the relative importance of various risk factors and to extend the national and regional findings to Florida. RQ 1: Is health services utilization (11 categories) among minority AD Medicare beneficiaries different from the majority white population? RQ 2: Are expressed difficulties in access-to-care among racial/ethnic minorities with AD different from the majority white population? RQ 3: Are the prevalence and mortality rates among racial/ethnic minorities with AD different from the majority white population? To carry out this proposed research study, the laboratory staff will use the Medicare Current Beneficiary Survey (MCBS-C&U) 2009-2012; which is a continuous, multi-purpose survey of a representative sample (~12,000) of the Medicare population, including aged and disabled enrollees. The MCBS-C&U file is a combination of self-reported survey data and respondent Medicare claims summaries. Survey questions cover health status and function, health insurance, household and facility characteristics, demographics (detailed race and ethnicity categorization) and cost and utilization provided by their Medicare claims and self-report for inpatient, outpatient, prescription drugs and facility use among others. Detailed questions about

activities of daily living, instrumental activities of daily living and caregiver status are also included, as well as behavioral issues like substance abuse, exercise, diet and weight. The analysis will estimate 11 measures of utilization e.g., inpatient, ambulatory, long term care, and prescription drug utilization, as well as across racial ethnic groups identified by the survey. Analysis will include cross-sectional analysis of each year as well as longitudinal analysis of the 2009 to 2011 data. Descriptive and predictive models will provide measures of racial/ethnic differences and to identify the relative importance of known risk factors. Study results will become the first step in a research agenda with the goal of conducting longitudinal access and utilization and disparities research using all Medicare beneficiaries from 1999 to 2013. Since AD and related disorders often progresses slowly, long-term, life course studies that track persons before the first diagnosis with many years of follow-up are more likely to elucidate causal patterns of the identifies disparities.

6. **Grant #8AZ06:** Quantitative Neuropathology And Biochemistry Of Survival Differences In Hispanic Americans With Alzheimer's Disease

   Principal Investigator: Melissa Erin Murray, PhD

   Organization: Mayo Clinic Jacksonville

   Abstract of Proposed Research: Risk of developing Alzheimer's disease (AD) dementia is 1.5 times greater in Hispanic Americans compared to European Americans, and twice as high in African Americans. Intriguingly, Hispanic Americans are found to live longer with the disease, suggesting that there may be resiliency factors currently unknown. With one of the largest series of autopsy-confirmed Hispanic Americans with an AD neuropathologic diagnosis (n=83) located at this institution, the research team is uniquely positioned to examine what changes in the brain may account for differences in survival. Using sophisticated technology to measure AD-related changes to proteins, the researchers will be able to examine what biological factors may differ between Hispanic Americans and European Americans. They will also provide exploratory comparisons with autopsy-confirmed African Americans with AD in a smaller cohort that is available (n=24). With a much larger cohort of European Americans (n=1796), the team will be able to match case-to-case for important factors, such as age at death, sex, and education. They will carefully review clinical history for measures of cognitive reserve by examining evidence of bilingualism and converting occupation to a job level score (as recommended by the Department of Labor and Statistics). Together this data will provide one of the first translational neuropathology studies to specifically examine survival of Hispanic Americans with AD.

7. **Grant #8AZ07:** Impact Of TREM2 Variants On Microglial Function And Alzheimer's Disease Pathology

   Principal Investigator: Chia-Chen Liu, PhD

   Organization: Mayo Clinic Jacksonville

   Abstract of Proposed Research: Emerging evidence showed that microglial activation is a beneficial response in the early phases of Alzheimer's disease (AD),

leading to increased Aβ clearance. However, at late stages of AD, microglia may paradoxically exacerbate the disease by secreting toxic pro-inflammatory cytokines in response to Aβ and other pathologies. Thus, understanding how microglia and neuroinflammation contribute to the disease development and progression may help determine the therapeutic window and strategy for introducing mechanism-based therapy for AD. Recent studies showed that a Arg-47-His (R47H) mutation of the triggering receptor expressed on myeloid cells 2 (TREM2) significantly increases AD risk by three-four fold. TREM2 is an innate immune receptor primarily expressed by microglia in the brain and is involved in inflammation and phagocytic clearance of Aβ and cellular debris. Although conflict data exist, TREM2 deficiency increases Aβ accumulation and neuronal loss in AD mouse models, suggesting that microglia may require TREM2 to respond to Aβ deposition and to limit neuronal damage. However, it remains unclear how AD-associated TREM2-R47H mutation affects microglial functions and amyloid development. Dr. Chia-Chen Liu and the research staff have recently developed novel mouse models expressing human TREM2 in an inducible, cell-type specific manner. After breeding to Cx3cr1-CreER mice, they generated microglia-specific TREM2 or TREM2-R47H mouse models in the Trem2-/- background. Using this unique model, the laboratory staff aim to dissect how expression of TREM2 and TREM2-R47H variant in microglia at different stages of amyloid pathology impacts cognition and amyloid pathogenesis. In this proposed study, they hypothesis that the AD-associated mutation, TREM2-R47H, impairs microglial functions, enhances pro-inflammatory responses and exacerbates amyloid pathogenesis, thus accelerating AD pathogenesis. The research team has established several innovative approaches, including in vivo two-photon microscopic imaging (for examining the microglial responses and amyloid development), and in vivo microdialysis (for measuring brain ISF Aβ and inflammatory cytokines) for this proposed study. In Aim 1, they will determine the effect of microglial TREM2 and TREM2-R47H on inflammatory responses, neuronal functions and behaviors. In Aim 2, they will induce the expression of TREM2 or TREM2- R47H in the background of amyloid model mice at different stages of amyloid development to examine their effects on brain Aβ metabolism, amyloid pathology, and Aβ-associated microglia activation. Effects on brain Aβ clearance and neuroinflammatory cytokines will be assessed by in vivo microdialysis, whereas effects on amyloid plaque and microglial responses will be examined by histopathological, microscopic, and biochemical methods. Together, this proposed study aimed at dissecting how TREM2-R47H modulates microglial functions and amyloid development, should provide mechanistic guidelines as to how microglia- mediated neuroinflammation can be targeted in AD therapy.

8. **Grant #8AZ08:** Evaluating The Impact Of A Dementia-Caring Community Model On African Americans With Alzheimer's Disease And Their Care Partners.

Principal Investigator: John A. Lucas, PhD

Organization: Mayo Clinic Jacksonville

Abstract of Proposed Research: Communities can play an important role in helping residents with Alzheimer's disease (AD) and their care partners obtain appropriate services and overcome the challenges and stigma that threaten quality of life, social well-being, and functional independence. Ethnic minority communities experience a disproportionately high degree of health disparities, including greater unmet needs and

increased barriers to dementia information and health care. For example, African Americans have a significantly higher prevalence of AD than Caucasians but typically do not present themselves to healthcare providers until much later in the disease course. A number of sociocultural factors have been identified to help understand this disparity, including lack of awareness of the early signs of AD, mistrust of the medical establishment, and limited access to clinical resources and caregiver support. The national plan to address AD encourages community engagement through the Dementia Friendly America (DFA) initiative of the National Alzheimer's Project Act (NAPA). This initiative provides a roadmap and tools to help systematically identify and implement opportunities to build 'dementia-friendly' or 'dementia-caring communities' (DCC). A DCC is a community where residents, businesses, and local governments work together to be supportive and inclusive of people with dementia in the places they live, socialize, worship, and work. To date, DCC efforts have been implemented in 36 US cities across 28 states.

A key component missing from the DFA toolkit, however, is the measurement of intended outcomes following program implementation. Although a number of qualitative studies related to the DCC concept and implementation have been published, there are currently no scientific studies documenting the impact of DCCs on community awareness or the health and quality of life of people living with AD and their care partners. Given the significant demands on time and monetary resources required to implement the DFA roadmap and maintain DCC programs, it is important to demonstrate objectively that these programs provide meaningful and measurable benefit. The aims of this proposal are to measure the impact of the DCC approach and DFA roadmap on community awareness and understanding of dementia, and on the quality of life of residents with AD and their care partners in a traditionally underserved African American community in Jacksonville, FL. Findings can help inform other communities with limited resources about the scope and magnitude of expected outcomes of DCC programs relative to cost, and can facilitate the implementation of DCC initiatives in ethnically diverse neighborhoods across Florida.

9. **Grant #8AZ09:** Targeting Lrrk2 Activity To Modulate Tau Pathology

Principal Investigator: Heather Melrose, PhD

Organization: Mayo Clinic Jacksonville

Abstract of Proposed Research: The link between leucine rich repeat kinase (LRRK2) and Alzheimer's disease (AD) pathology is intriguing. Brains with LRRK2 mutations can display pleomorphic pathology including tau and amyloid inclusions. Novel tau epitopes phosphorylated by LRRK2, and overexpression of human wild-type LRRK2 in mice promotes tauopathy. Several Rab GTPases, including Rab3, have been identified as in vivo LRRK2 substrates. Expression changes of Rab proteins and their effectors are found in post-mortem AD brain and effector rab3- GEF was recently nominated a modifier of tau toxicity in a genome-wide association meta-analysis for AD. It is proposed that overactive LRRK2 disturbs intracellular trafficking via promoting the dissociation of GDIs (guanine dissociation inhibitors) in the cytosol with concomitant membrane insertion, altering the relative pool of membrane bound and cytosolic Rabs. The research team suspects that the unsuccessful aging process in humans could be due to the cellular localization of LRRK2 gradually altering and thus increasing the

likelihood of this unwanted action of LRRK2. To examine the relationship between LRRK2 and tau, the researchers expressed AAV-human tau on a LRRK2 G2019S mutant, knockout (KO) or wild-type background. Loss of LRRK2 increased survival of AAV-tau mice, despite reaching the same stage of Ab39- positive mature tangle pathology as the G2019S or WT mice expressing AAV-tau. Surprisingly, KO/tau mice had significantly more soluble phospho-tau than G2019S/tau or WT/tau mice, suggesting an alternative species of tau is the toxic mediator. They suspect that LRRK2 activity may have an unsolicited role in the spread of tau oligomers via rab protein signal regulation. Interestingly, it has recently been shown that worms expressing tau A152T mutant, a risk factor for fronto-temporal dementia, have cargo transport deficits associated with mislocalized Rab3a.

The researchers hypothesize that reducing LRRK2 levels will restore the Rab pool distribution and reduce abnormal trafficking of tau. LRRK2 is an attractive drug target, bearing a dual enzymatic core consisting of a kinase and GTPase domain. Drug companies have invested heavily in LRRK2 therapeutic programs and it envisaged that LRRK2 therapy could extend to neurodegenerative diseases like AD. As well as targeting enzymatic activity, there is also precedence for lowering LRRK2 levels. For example, LRRK2 silencing in rodents protects against inflammatory brain insult via lipopolysaccharide and α-synuclein-induced dopaminergic degeneration. For this proposal, the research team will test the effect of a LRRK2 inhibitor MLi2 (Merck) and a LRRK2 antisense oligonucleotide (ASOs, Ionis) in a mouse model of tauopathy that expresses low levels of human wild-type tau and in adulthood has tau seeding initiated via inoculation of tau filaments isolated from AD brain. Outcome measures in mice will be behavior, neuropathology and biochemistry. If successful, this work will underline a role of LRRK2 in AD with a focus on regulating intracellular trafficking and Rab GTPase biology. They hope to define early molecular changes related to tau spreading and which of these alterations might be reversible. Data to support a beneficial role of LRRK2 modulation in AD mouse models may also nominate biomarkers for disease diagnosis and progression/reversal monitoring.

10. **Grant #8AZ10:** Identifying Drug Targets Using Long-Read Sequencing In Alzheimer's Diseased And Control Brain Tissue

Principal Investigator: Mark T. W. Ebbert, PhD

Organization: Mayo Clinic Jacksonville

Abstract of Proposed Research: Alzheimer's disease (AD) etiology, diagnostics, and effective treatments have eluded researchers for over 100 years because the disease is remarkably complex, but with new technologies at our disposal, the potential to identify the underlying disease biology can be attainable. Researchers have implicated many genes through extensive efforts and collaborations, including the Alzheimer's Disease Sequencing Project (ADSP), but given the impending healthcare crisis, we need to direct efforts to understanding disease etiology under the best circumstances by studying the diseased tissue using the best technologies. Dr. Mark Ebbert and research staff seek to perform deep, targeted long-read DNA sequencing and deep, targeted long-read RNA isoform sequencing (IsoSeq) in the lateral entorhinal cortex using PacBio long-read technology, which is best- suited for identifying structural mutations and accurately sequencing individual RNA isoforms. This approach provides a clear path to

understanding a crucial aspect of AD etiology by identifying structural mutations that may be the functional mutations associated with genome-wide association hits researchers have been looking for. The approach of this study will also enable the researchers to identify aberrant RNA isoforms that drive disease development and progression. By studying the lateral entorhinal cortex, where pathogenesis typically begins, they can maximize the likelihood of finding any mutations involved in disease, ultimately leading to effective therapeutics. It is critical to study these avenues in the diseased tissue, because genetic mosaicism is evident across tissues. With a strong research team, along with collaborators at Integrated DNA Technologies (IDT) and Pacific Biosciences (PacBio), this team plans to provide existing evidence that supports their aims while also helping advance AD research.

11. **Grant #8AZ11:** Impact Of The Modified MindSet Training Program On Maintaining Optimal Function Among Early Alzheimer's Patients And Their Care Partners

Principal Investigator: Sindy Goenaga, MD, MPH

Organization: Mount Sinai Medical Center

Abstract of Proposed Research: The Wien Center for Alzheimer's Disease and Memory Disorders proposes to implement and to assess the impact of a novel modification of the Mindset Training Program. This pilot project is very much aligned with the goals of the Florida Department of Elder Affairs Alzheimer's Disease Initiative and its Dementia Care and Cure Initiative. During this grant period, the research staff plan to address focus area 1.2 which is the social environment of persons with Alzheimer's disease and related dementia (ADRD), and focus area 5.3 as it addresses novel diagnostic procedures, tools, and strategies. The aims of the novel project are: 1) to implement the Revised MindSet Training Program, 2) to assess and measure the following outcomes: overall satisfaction with this training program, effects of the program on communication between the care recipient and their care partner, effects of the program on patient cognition and function in both English and Spanish speaking individuals. This program will be implemented over the next two years and will target persons with Mild Cognitive Impairment (MCI) and early stage dementia. The MindSet training program was originally developed as a 6-week course, based upon a study program conducted by Dr. David Lowenstein at the University of Miami. The curriculum was developed to be used in small groups in a classroom format, with an emphasis on participants will learn exercises to better maintain their functional abilities and to develop strategies to better use the cognitive skills they currently possess. In this proposal, there will be an increased focus on improving attention, enhancing cognitive processing speed, spaced retrieval, procedural memory, and other techniques that engage cognitive functions which are not greatly dependent on memory. Further, there will be efforts to assist the care provider with enhanced communication skills, stress management techniques and behavioral skills that should optimize quality of life for both the caregiver recipient and care provider, enhance communication skills and strengthen the caregiver and care recipient partnership. This novel project will provide persons with MCI and early stage dementia with tools which will allow them to maintain or prolong independence and functionality as well as a means to build social support networks and improve communication and to increase quality of life. A unique aspect of this program as compared to the original Mindset program is a dual focus on both care recipients and care providers in supportive small group settings. It is expected that the caregivers or

study partners of the participants will also benefit from this training program by allowing them to be active agents in improving the quality of life of the patients and to reduce caregiver stress.

12. **Grant #8AZ12:** Protein Disulfide Isomerase Uses Conditional Disorder As A Disaggregase Mechanism To Detoxify Amyloid Beta Fibrils

    Principal Investigator: Ken Teter, PhD

    Organization: University of Central Florida

    Abstract of Proposed Research: In Alzheimer's disease (AD), neurotoxic aggregates of amyloid-beta (Aβ) peptide damage the brain. Protein disulfide isomerase (PDI), which is produced by most cells in the human body, can prevent the aggregation of Aβ. An S-nitrosylated form of PDI that cannot prevent protein aggregation is found in the brains of individuals with AD. PDI has also been found embedded in aggregated Aβ plaques. In recent studies, the research team has identified a novel property of PDI that could be linked to its protective role in AD. They have shown PDI will unfold upon contact with aggregated Aβ, which provides a possible molecular mechanism for the disruption of protein aggregation by PDI: unfolded proteins are larger than folded variants of the same protein, so the expanded size of unfolded PDI would push against two peptides in the Aβ aggregate and consequently act as a wedge to displace individual peptides from the aggregate. PDI could thus reverse, as well as prevent, Aβ aggregation. In support of this model, the researchers have shown PDI can dissolve neurotoxic aggregates of α-synuclein, a protein that contributes to Parkinson's disease. Preliminary data indicate PDI can also protect neuronal cells from the toxicity of aggregated Aβ.

    For this project, the researchers will define the structural events that accompany the Aβ-induced unfolding of PDI and its resulting disaggregase activity that dissolves and detoxifies amyloid fibrils. This work will provide detailed mechanistic insight into the unique and previously unrecognized disaggregase activity of PDI that could identify recombinant PDI as a potential treatment for amyloid-induced neurodegeneration.

13. **Grant #8AZ13:** Optical Characterization Of The Aggregation (Change in size, Fibril Formation), Accompanying Structural Changes, And Membrane Pore Formation.

    Principal Investigator: Florencio Hernandez, PhD

    Organization: University of Central Florida

    Abstract of Proposed Research: Alzheimer's disease (AD), the most common form of dementia in senior citizens is among the top six leading causes of death in the USA, and the number of cases is projected to triple by 2050. Because of its irreversibility, AD poses a large financial and social burden in families and society. Therefore, finding ways to prevent, stop the progression and cure AD is a vital priority. For this purpose, a better understanding of the pathophysiology of AD need to be achieved. It is currently known that AD is characterized by the extracellular deposition of amyloid plaques in the cerebral neuropil and vasculature, and the accumulation of intracellular neurofibrillary tangles. However, there is not solid consensus on the species of Aβ peptides that exert

the major neurotoxic effect, e.g. while the original amyloid hypothesis suggested that the degree of accumulation of insoluble fibrils of amyloid determines the extent of neurotoxicity, recent evidence support the role of soluble amyloid peptides and oligomer aggregates as the main neurotoxic effectors. In addition, besides the aggregates size, the cytotoxic effect of Aβ seems to be determined by its molecular conformation. However, more fundamental structural studies are needed to establish a better correlation between the specific structural characteristics of Aβ and their neurotoxic effect.

The research team propose a novel approach for the structural analysis of different species of Aβ that will lead to a better understanding of the process of Aβ aggregation and fibrillation, and membrane pore formation potentially involved in AD. Throughout this project, the research staff will combine two-photon circular dichroism (TPCD) and isotope-edited FTIR (IE-FTIR) to tackle the structural distortions underlying the aggregation of full- length Aβ and several fragments that represent structurally distinct and functionally important stretches of the peptide. The high sensitivity of TPCD to small structural distortions and its capability to access specific conformational fingerprints in a region of the electromagnetic spectrum inaccessible by any other means (vacuum ultraviolet), synergistically working with the characteristic site-specific resolution and sensitivity of IE- FTIR to small differences in intra- and interstrand H-bonding in β-sheets, guarantee the access to specific conformations and structural distortions of specific peptide oligomers (Aβ11-28, Aβ25-35 and Ab1-42) that can result in the formation of amyloid plaque seeds. This approach will overcome some of the limitations presented by the techniques most commonly used for structural analysis. The findings of the proposed research will have a strong impact on fundamental research of AD.

14. **Grant #8AZ14:** Factors Influencing Family Caregivers' Medical Decision-Making For Patients With Advanced Alzheimer's Disease

Principal Investigator: Elzbieta Sikorska Simmons, PhD

Organization: University of Central Florida

Abstract of Proposed Research: The goal of this 12-month prospective study of 20 family caregivers for 20 home-dwelling patients with advanced Alzheimer's disease (AD) is to gain an understanding of the ways they manage the disease. Specifically, the aim of this project is to examine the effects of advance directives and other factors on caregivers' decisions to seek or discontinue medical treatments when faced with potentially life-threatening symptoms, co-morbid conditions, and acute medical events. AD is a progressive, terminal illness with a median life expectancy of three to twelve years from the time of diagnosis. Most of these years are spent in advanced stages that require increasingly higher levels of care. Family caregivers care for the majority of these patients at home and gradually become surrogate decision-makers. Most patients die in nursing homes, but home deaths increased from 14% in 1999 to 25% in 2014. Home clinical management of advanced AD poses special challenges because of complex symptoms, co-morbid conditions, and acute medical events like pneumonia that can be fatal if medical treatment is withheld. Results from research conducted in nursing homes have shown that the length of patient survival is greatly influenced by the caregiver's decisions to seek or decline treatment. Little is known about how caregivers make these decisions and choose treatments for home-dwelling AD

patients. Even less is known about the implications of their decisions for patient quality of life, caregiver burden, and utilization of medical services over time.

The naturalistic decision-making model, which focuses on the role of personal and situational factors in making decisions, will provide a conceptual framework for this analysis. Based on the literature, these factors can be classified into six categories: 1) advance directives (e.g., living will) 2) patient- related (e.g., co-morbid conditions), 3) caregiver-related (e.g., knowledge about dementia), 4) health professionals-related (e.g., primary care physician); 5) access to services (e.g., palliative care); and 6) social context (e.g., family). The objective of this study is to identify pathways through which these factors influence caregivers, medical decision-making and examine implications of these decisions for patient quality of life, caregiver burden, and utilization of health services. A convenience sample of 20 caregiver/patient dyads will be selected from a list of Dr. Laird's (Co-Investigator) patients at Center for Senior Health in Winter Park, Florida. The following criteria will be used in the sample selection: 1) the patient must be 65 or older and diagnosed with AD, 2) the family caregiver must be his/her legal health care proxy, and 3) the patient must reside at home. Caregiver interviews will be conducted in person every four months. Quantitative and qualitative data will be collected using a semi-structured interview schedule. The interviews will last approximately 30-45 minutes. All interviews will be taped and transcribed. In addition to qualitative questions, scales will be used to assess caregiver burden, knowledge about dementia, and utilization of services. The qualitative data will be analyzed for themes. To accomplish this, the data will be analyzed through a series of regression models to examine the relationships between all variables delineated in the study's conceptual framework.

15. **Grant #8AZ15:** Periodontal Bacteria Augment Progression Of Abeta; And Tau Pathology

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

Organization: University of Florida

Abstract of Proposed Research: Multiple epidemiological, clinical and molecular studies have shown that chronic gum (periodontal) disease (PD) in the mouth associated chronic inflammation (co-morbidity or cofactor) is linked with increased risk and progression of varying forms of dementia, including Alzheimer's disease (AD). AD is a complex disease of unknown etiologic origin with both environmental and genetic risk factors, contributing to its onset. Systemic and brains local inflammation precedes neurodegenerative processes and predicts clinical onset of AD. Many studies suggested that high burden of host inflammation maybe responsible for amyloidogenic processes in AD, at least in preclinical mice models. Fibrillary amyloid beta (Aβ) is central to the AD neuropathology, is neurotoxic, and has the capacity to inappropriately activate the innate immune responses including release of several cytokines. However, susceptibility to peripheral microbial infections appears to increase during advancing age with the innate immune system becoming inadvertently activated as a disease promoting factor. Gum disease, which is one of the most common forms of chronic infection among adults, is characterized by loss of tooth supporting tissues and complex bacteria underneath the gums. Numerous studies link gum disease associated chronic inflammation with increased risk of dementia, including AD. Plasma levels of antibodies to gum disease bacteria are significantly higher in AD patients. One study directly

demonstrated the presence of seven different oral motile spiral bacteria (spirochetes), causative of gum disease, in autopsy brains from AD subjects. This is consistent with the researchers' findings that another significant oral bacterium, Porphyromonas gingivalis lipopolysaccharide (LPS) present in four out of ten AD brain tissues and subsequently the innate immune activation is a critical risk factor for developing AD. Following chronic infection of mouse gingival cavity with P. gingivalis, the research team also detected the bacterial genomic DNA and viable bacteria in brains of infected mice. In spite of these findings, the mechanism by which gum disease may be considered a risk factor for specific pathological hallmark proteins found in AD remains obscure. Despite interesting links between AD and periodontitis and growing evidence that oral bacteria can regulate pathological hallmarks of AD, there is no in vivo study targeting a role for oral bacterium in the initiation of Aβ plaque and Tau pathology in mouse models of AD-like amyloidosis.

The proposed hypothesis is that chronic systemic inflammation caused by the oral bacterium infection increases amyloid aggregation and neurofibrillary tangle (NFT) pathology in the AD models and enhances neuroinflammation and neuronal injury. The specific aims are to explore the role of several oral bacterial infection in regulating Aβ and tau pathology in TgCRND8 (aggressive Ab plaque transgenic mouse), MHSI-695-GFP#3 (a non-depositing AD transgenic mouse line), JNPL3 (hyperphosphorylated tau NFT formation) mouse models. The objective is to determine the possible causal link between oral bacteria with AD. The long-term goal is to investigate the mechanistic basis of how CNS and/or systemic inflammation affect AD proteostasis and determine therapeutic strategies. This is a multidisciplinary project between laboratories that has expertise in gum disease (Dr. Lakshmyya Kesavalu) and neuroinflammatory mechanisms of neurodegenerative diseases (Drs. Todd Golde and Yona Levites).

16. **Grant #8AZ16:** Towards Understanding The Biological Role Of Newly Discovered Alzheimer's Disease Susceptibility Genes Affecting Immune Function

    Principal Investigator: Paramita Chakrabarty, PhD

    Organization: University of Florida

    Abstract of Proposed Research: Gliosis, reflective of the underlying alterations in microglial function, is a pathological feature of Alzheimer's disease (AD). Recent genetic and transcriptomic data have identified several microglial genes that are part of an innate immune network that is associated with increased risk of AD. Cumulatively, gene expression data from various laboratories, including this institution, suggests that altered immune response observed in AD may have a direct role in the pathogenesis of amyloid β plaques and tau tangles (two hallmark pathologies in Alzheimer's disease). This research group, as part of the NIH AMD-AD consortium, has recently identified coding variants of two novel microglia-specific genes, ABI3 and PLCG2, that confer significant risk for Alzheimer's disease. The ABI3 gene variant (rs616338:p.Ser209Phe) increases the risk for AD where the PLCG2 variant (rs72824905:p.Pro522Arg) is a protective variant. Protein-protein network analysis places both ABI3 and PLCG2 in an immune network encompassing two other Alzheimer's related microglial genes, TREM2 and SPI1. This strongly suggests a functional role for both ABI3 and PLCG2 in the Alzheimer's pathological cascade. However, the exact biological mechanisms

underlying ABI3 and PLCG2 mediated events that alter microglia function and Alzheimer's pathogenesis is unknown.

In this proposal, the research staff have devised experiments that will help them understand the role of these two novel genes, ABI3 and PLCG2, in AD pathological cascade. They will first generate mouse models of Alzheimer type amyloidosis that is deficient in either ABI3 or PLCG2 to assess how these genes affect Alzheimer-related Neuropathologies. Using primary microglial cultures or brain slice cultures generated from different mouse lines, they will investigate how deficiency of ABI3 or PLCG2 proteins or overexpression of Alzheimer-associated variants of ABI3 and PLCG2 proteins affect amyloid β catabolism. Knowledge from this project can help shed light on how microglia-mediated immune response contributes to AD pathogenesis and further help in designing the next generation immunotherapeutics that can be used effectively as therapies in AD.

17. **Grant #8AZ17:** Precision Public Health Approaches To Reduce Disparities In Memory Disorder Screening In Rural Minority Communities

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

Organization: University of Florida

Abstract of Proposed Research: Reducing health disparities for Alzheimer's disease (AD) in mortality among Florida's diverse, aging, population is the focus of this proposal. Specifically, it will overcome barriers to accessing Florida's Memory Disorder Clinics. These barriers are especially relevant to rural and African-American populations 60 years of age and older in areas with the highest AD age-adjusted death rates in Florida. Barriers include: poor access to care, which complicates AD screening, diagnosis and treatment. Other barriers are at the community level (lack of public awareness), service provider level (insufficient specialty care, reluctance to diagnose, and inadequate capacity to screen) and individual and family levels (stigma, embarrassment, expensive treatment, a perception that care is ineffective and difficult to access, lack of knowledge about where to seek help, and denial). Building on the successful and innovative community engagement model called HealthStreet, this project will empower Community Health Workers (CHWs), at the center of their model, to reduce these barriers. While the geographical areas that are being focused on have had the highest AD mortality rates, they have had the lowest AD case rates, suggesting a critical lack of screening. This is primarily due to a lack of knowledge, misperceptions of primary care physicians on consequences of undiagnosed and untreated AD, and not knowing how to refer to Memory Disorders Clinics.

For this proposal, residents from eight North and North Central Florida counties (population = 1,013,693; 193k >65 y/o) with the highest mortality from AD with one comparison site (Alachua) will be recruited: Bay, Calhoun, Franklin, Gulf, Holmes, Jackson, Liberty, Marion, Putnam, Wakulla and Washington). From 12 to 100% of these counties are rural; from 8% to 28% are African-American. From 14% to 29% are below the poverty level. Specifically, CHWs will assess 2,000 community members 60 years of age and older for cognitive status, Alzheimer's disease knowledge, risk factors, social determinants of health and health histories; they will then be screened in the community with the Montreal Cognitive Assessment (MoCA). Based on their health concerns and

needs, residents will be referred to medical and social services and be given referrals to further cognitive screening through their physician. The physician will be given educational materials on how to refer their patient to a local Florida Memory Disorder Clinic. CHWs will follow up with each person at 60 and 120 days to evaluate these metrics of success: screening referral, completion of screening, barriers, and increased knowledge of AD resources, consequences and symptoms among at risk community members, their physicians and caregivers. The researchers anticipate that this project will improve recognition of AD in counties with large discrepancies between AD mortality and AD case rate.

18. **Grant #8AZ18:** Investigations Of Neuropathologies Targeted By Clinical Trials In Alzheimer's Disease Patients

Principal Investigator: Anthony T. Yachnis, MD

Organization: University of Florida

Abstract of Proposed Research: Patients with Alzheimer's disease suffer from accumulation of pathological proteins including ABeta- amyloid and tau. These abnormal proteins are components of senile plaques and neurofibrillary tangles, respectively, which are the defining neuropathological findings in the disease. Many patients with Alzheimer's disease also have ABeta-amyloid accumulation in brain blood vessels, which predisposes them to hemorrhage or stroke. Multiple recent clinical trials have employed drugs that target ABeta- amyloid and tau, in order to inhibit or reduce accumulation of the abnormal proteins and curtail disease progression. To provide new insights in the contribution of specific Alzheimer's disease pathological proteins and how these are altered by specific therapeutic targets, the research team proposes to uniquely assess the brain pathologies from patients who have been the subjects of novel therapeutic treatments primarily at the Compass Clinic, Orlando. Compass has been involved in over 245 neurological trials in all phases and has enrolled over 10,000 participants in neurology alone. These patients will be extensively assessed for pathological findings using consensus guidelines from the National Institute of Aging/Alzheimer's Association, as well as novel state-of-the-art reagents and tools developed by the 1Florida Alzheimer's Disease Research Center (1Florida ADRC). This research project will provide new insights into the effects of these therapies on ABeta or tau accumulation, while providing information on the relative role of specific brain pathologies driving the etiology of Alzheimer's disease.

19. **Grant #8AZ19:** Role Of Microglia In Primary Age Related Tauopathy And In Sporadic (Late-Onset) Alzheimer's Disease

Principal Investigator: Wolfgang J. Streit, PhD

Organization: University of Florida

Abstract of Proposed Research: The most common form of Alzheimer's disease (AD) accounting for more than 95% of all cases is called sporadic or late-onset AD (LOAD). Sporadic means that there are no clearly identifiable genetic abnormalities associated with it, and the disease can therefore affect anyone. Not only does it remain unknown

why some people are more likely to develop LOAD, it is not even known or agreed upon by neuroscientists how exactly the disease evolves in an individual, i.e. the pathogenesis is incompletely understood. Obviously, an understanding of pathogenesis is essential for developing effective treatments. The best way to study LOAD pathogenesis is in human brains because LOAD is a uniquely human condition that laboratory animals cannot represent. Neuropathological studies have shown that the microscopic disease process, characterized by distinct lesions, begins as early as childhood and continues to gradually progress over subsequent decades until it eventually turns into LOAD. During much of this time, and as the microscopic pathology slowly worsens, individuals do not experience any problems with memory or cognition: clinically, they are considered normal (non-demented). If any of these individuals come to autopsy at this stage, a diagnosis of preclinical Alzheimer's can be made only after a neuropathologist has studied the brain and has found the characteristic lesions. However, it is still unknown at what point the pathologic changes in the brain have progressed sufficiently to cause clinical problems; in other words, how much pathology does it take to produce symptoms? The answer to that question means a better understanding of the disease process and therefore being able to devise effective treatments.

The aims of this project are to perform neuropathological studies on a random sample of thirty individuals who at the time of their deaths were non-demented. For each individual, the research team will examine the same brain regions (entorhinal cortex, hippocampus) identifying lesions that are characteristic of LOAD. They will use five-six specialized histological (immunohistochemical) procedures that will allow them to identify clearly Alzheimer- type lesions. The research team will pay particular attention to cells that make up the brain's immune system (microglial cells), as it is believed that microglia are critically important for the LOAD process. The researchers expect that by examining in thirty individuals all of the three pathological hallmark features that are currently recognized to be important for understanding LOAD pathogenesis (plaques, tangles, and microglial activation), they will be able to significantly illuminate the chain of microscopic events that eventually results in dementia.

20. **Grant #8AZ20:** Seeded Interactions Of Abeta; And Neurofibrillary Tangle Pathologies In Mouse Models

    Principal Investigator: Guilian Xu, PhD

    Organization: University of Florida

    Abstract of Proposed Research: Although many transgenic mouse models exist that develop senile amyloid-beta (Aβ) plaques and neurofibrillary tangles, it is not clear that any of the existing models show robust interdependency in which Aβ pathology influences tau inclusion formation. There have been multiple recent studies indicating that both Aβ and tau pathology can be independently accelerated in these models by injecting tissue preparations that contain high levels of misfolded Aβ or tau, respectively. These acceleration models produce animals in which pathology develops within 6 months of injection, or less, instead of taking 12 months or more. For example, this institution recently developed such a model system based on mice that express humanized amyloid precursor proteins encoding mutations linked to early onset Alzheimer's disease (AD; APPswe/ind). These mice, termed PrP.APPsi express the

transgene at a level that causes amyloid deposition beginning at 12-14 months. When human AD brain lysates are injected into the brains of newborn (postnatal day 0) PrP.APPsi mouse pups (P0 injection), the researchers dramatically accelerated Aβ deposition such that a 12-month-old injected mouse had the level of amyloid pathology observed in 20-month-old uninjected mice. Thus far, the research team has observed this outcome using inocula from several independent human AD cases. Similarly, Aβ deposition can also be dramatically accelerated by injecting brain homogenates prepared from transgenic mice with Aβ aggregates that express either human or mouse Aβ. A similar phenomenon is observed using mice overexpressing the P301S mutation of human tau and driven by the mouse PrP promoter (PS19), which develop spinal cord pathology beginning ~ six months of age and succumb to paralysis between 8-12 months of age. Numerous studies have demonstrated that intracerebral injections into PS19 mice with in vitro synthesized tau fibrils, or mouse and human lysates containing tau inclusions, can induce the earlier formation of neurofibrillary tangles.

Together, these data demonstrate the ability for exogenously applied preparations of both Aβ and tau to enhance and accelerate the disease process in these transgenic mouse models. In the current proposed study, the researchers hypothesize that seeding double transgenic mice expressing both human APP and P301S tau with inoculum containing one or both of these proteins will establish an accelerated mouse model that develops both Aβ plaques and neurofibrillary tangles before six months of age. If successful, such a model would be very useful in pre-clinical studies to identify therapies that slow the formation of Aβ and tau pathology.

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

Principal Investigator: Rosie E. Curiel, PsyD

Organization: University of Miami Miller School of Medicine

Abstract of Proposed Research: The focus of this research fellowship study is to offer a promising postdoctoral candidate the opportunity to receive specialty training and develop skills in research methodologies used to clinically assess diverse older adults and individuals on the Alzheimer's disease continuum. Drs. Rosie Curiel and David Loewenstein, who would serve as primary and secondary mentors at the University of Miami Center on Aging, have expanded their program of research that will host the training for the postdoctoral fellow. This training environment includes an ongoing longitudinal NIH RO1 on aging and cognition (Loewenstein-principal investigator (PI)), and a new RO1 (Curiel-PI) which easily achieved the NIH pay line and focuses on state-of-the-art computerized assessment for the detection of preclinical AD. Moreover, the research team also has two ongoing Ed and Ethel Moore research studies and leads a major scientific project at the 1Florida ADRC at Mt. Sinai Medical Center and provides all neuropsychological assessments for the UM Memory Disorders Clinic. Postdoctoral training also offers a strong focus on cross-cultural neuropsychological assessment and the development of culturally fair diagnostic assessment instruments, which is of critical relevance in the State of Florida. Taken together, this focused and highly productive program of research led by Drs. Curiel and Loewenstein, along with their longstanding background in academic training, serve to offer prime training opportunities for the postdoctoral candidate to expand their competency to serve minority older adults who are at risk for the development of neurodegenerative conditions such as AD.

This institution is the only academic medical center in south Florida that can offer a postdoctoral research fellow the platform to cross-train on multiple Alzheimer's disease federally funded research studies at the UM Center on Aging. This, along with their collaboration with the 1Florida Alzheimer's Disease Research Center, will offer an unparalleled specialty training opportunity. Competent cognitive assessment that is sensitive to detect PreClinical AD remains a critical priority area in Alzheimer's disease research. Offering this training opportunity to a neuropsychologist is of particularly high impact, in that this discipline plays a direct and critical role in Alzheimer's disease clinical research. In addition, the longitudinal nature of this institution's research program will offer the unique opportunity for the fellow to assist with longitudinal data analysis, expose them to state-of-the-art cognitive assessment methods and various biological markers of AD pathology. Finally, the fellow will receive training in writing federally funded grants to prepare him/her to become an independent investigator.

22. **Grant #8AZ22:** Cardiovascular And Lifestyle Stressors Of Hippocampus And AD Related Brain Regions

Principal Investigator: Noam Alperin, PhD

Organization: University of Miami

Abstract of Proposed Research: Accelerated loss of brain tissue, especially in regions within the medial temporal lobe, is already apparent in preclinical AD. The proposed aim is to better understand how and why these cognitively critical regions are affected by external stressors. This multidisciplinary team has a unique track record in novel cognitive stress testing for early detection of AD and in neuroimaging using quantitation of brain structures and hemodynamics. This research team has a productive collaboration on an NIH R01 study (PI Loewenstein) that focuses on early cognitive and biological markers of preclinical AD. Team members have completed recruitment and MRIs for nearly 250 community- dwelling older adults ages 60-90. Their project will take advantage of this tremendous resource to measure the magnitude of cardiovascular and lifestyle stressors and their impact on AD-vulnerable brain regions. The MRI protocol included two novel methodologies that will provide a more refined brain parcelation (e.g., hippocampus sub regions) and measurements of cerebral blood flow dynamics. These novel MRI scans will be analyzed to establish the role of two related stressors that impact the health of these AD vulnerable brain regions: 1) sleep quality, and 2) cerebral vascular flow pulsatility. The new analyses were not part of the aims of the ongoing NIH study.

Sleep quality significantly impacts brain health. Recent publications suggest that removal of toxins from the brain through the cerebral spinal fluid (CSF) circulation occurs primarily during sleep. Thus, impaired sleep may be a risk factor for accelerated cognitive decline and AD through inefficient maintenance of the brain tissue. In a small cohort of cognitively intact elderly subjects the researchers found that subjects with poor sleep quality (not related to sleep apnea) had significantly smaller AD vulnerable regions (e.g., hippocampus [primarily left hemisphere], right inferior-parietal regions, left middle-temporal) compared to good sleepers. This project proposes to perform a more refined brain parcelation of the middle temporal lobe and the hippocampus sub-regions to test

for a link between sleep pattern of brain volume loss. This study will also attempt to assess the surrounding CSF spaces as estimates of toxin clearance efficacy.

Dementia is associated with endothelial and blood-brain barrier dysfunction, i.e., the tight junctions between endothelial cells lining the vessels that prevents toxins and large molecules from entering the brain. Previously, the researchers have found in a small cohort of patients with cognitive impairments that measures of vascular pulsatility have predicted the accelerated atrophy in AD vulnerable brain regions. They hypothesize that aging related deterioration in the ability of the cerebral vasculature to moderate the blood flow pulsation (due to loss of cerebral vascular mechanical compliance) causes expansion of the cerebral microvascular during each cardiac cycle, which in turn, stresses the tight junctions and causes weakening of the tight junctions. Use of this grant will help study a small cohort of younger healthy subjects to establish age related normative values of the cerebral vascular pulsatility measures as a normative reference. The success of this study will provide new knowledge that will lead to the development of interventions to reduce the potential harming effects of these stressors.

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

Principal Investigator: David Loewenstein, PhD

Organization: University of Miami

Abstract of Proposed Research: This proposed consortium between the University of Miami, University of Florida, Florida International University and Mount Sinai Medical Center, will generate an unprecedented opportunity to study state-of-the-art multi-modal neuroimaging (amyloid, tau, cortical thickness, regional brain volumes) and novel cognitive markers of early Alzheimer's disease among diverse ethnic and cultural groups (African-American, Hispanic and White-Non-Hispanics) at risk for Alzheimer's disease. This consortium will be the first in the State of Florida to examine the relationship between tau and amyloid load in the brain as they relate to novel cognitive stress tests that have been found to be extremely sensitive markers of PreClinical AD by uniquely tapping susceptibility to proactive semantic interference (PSI) and failure to recover from PSI (frPSI). The consortium will leverage existing resources and data provided by the 1Florida ADRC and the University of Miami's longitudinal NIH study on aging and cognition (Dr. Loewenstein, PI) and will recruit additional minority older adults at risk for AD.

The proposed consortium will also leverage the complimentary expertise offered by Drs. David Loewenstein and Rosie Curiel (University of Miami), Dr. Steven DeKosky (University of Florida), Dr. Maria Grieg (Mount Sinai Medical Center) and Dr. Malek Adjouadi (Florida International University). This collaboration provides special expertise in quantitative multimodal neuroimaging, diagnosis of early cognitive impairment (MCI and PreMCI states), and the development of novel cognitive stress paradigms that are cross-culturally sensitive. The goals of this consortium are to 1) determine whether ethnically diverse older individuals who exhibit PSI or frPSI deficits are at greater risk for AD related pathology (amyloid and tau load in AD prone areas) and 2) to relate these predictors to more ubiquitously available imaging measures such as cortical thickness and brain volumes in AD prone regions (e.g., hippocampus, entorhinal cortex,

precuneus, posterior cingulate). This consortium integrates unique expertise from three productive and outstanding institutions, focuses on state-of-the art-multimodal neuroimaging methods used to detect preclinical AD, leverages excellent diagnostic work-ups in African-American, Hispanic and White Non-Hispanic elderly as well as existing MRI, novel cognitive stress tests and amyloid data. Such a study is of high impact in that it expands upon and further refines diagnostic strategies for early detection of PreClinical AD and emerging treatments. It will also yield important pilot data for successful collaborative R01 and other federal grant submissions to the National Institutes of Health.

24. **Grant #8AZ24:** Extracellular Vesicles As Novel Therapeutic Targets In Alzheimer's Disease

    Principal Investigator: Michal Toborek, MD, PhD

    Organization: University of Miami

    Abstract of Proposed Research: Virtually all cells of the human body shed vesicles into the extracellular space, which then travel via the blood stream and can reach distant organs. These vesicles, named extracellular vesicles (ECV), carry content characteristic to the cells they originate from, including a protein called amyloid beta (Aβ). This study will propose that ECV can bring Aβ from the periphery into the brain by crossing the blood-brain barrier (BBB), a critical interface built by the brain microvessels, which normally protects the brain from blood-borne factors. Moreover, evidence suggests that this process is accelerated in Alzheimer's disease (AD), and contributes to Aβ accumulation in the brains of individuals suffering from AD. Increased deposition of Aβ in the brain is of critical significance because it generates pathology that involves memory loss and cognitive decline in AD individuals.

    The link between elevated Aβ levels in the brain and loss of memory in AD is not fully understood. However, it is important to note that neural progenitor cells (NPC), i.e., cells that produce new neurons even in the adult brain, are located in close proximity to brain microvessels forming the BBB. NPC-derived neurons are critically important for normal brain function because they are built into normal neuronal networks and participate in memory formation. This proposal will explore the role of ECV in Aβ transfer to NPC and the outcomes of this process, such as impaired production of new neurons, resulting in memory loss. The central hypothesis of the proposal is that Aβ carried by ECV across the BBB impairs formation of new neurons from NPC, resulting in memory loss. Specific mechanisms evaluated in this application include studies on the uptake of ECV and delivery of Aβ cargo to NPC (Aim 1), the impact of this process on the formation of new neurons from NPC (Aim 2), and alterations of cell-cell communication between NPC (Aim 3). The studies proposed in this project are pre-clinical and involve cell cultures and animal experimentations. Overall, this proposal offers a unique perspective on the interactions between the BBB, ECV, and Aβ deposits on impaired formation of new neurons in adult brain. The expected outcome of this project is that therapeutic targeting of this process can protect against Aβ pathology and memory loss in AD.

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

Principal Investigator: Anthony Griswold, PhD

Organization: University of Miami

Abstract of Proposed Research: Alzheimer's disease (AD) is the leading cause of dementia in the elderly and its prevalence is on the rise. Recent genome wide association studies (GWAS) have identified at least 20 genetic markers associated with AD. Unlike known autosomal dominant pathogenic mutations in genes such as amyloid precursor protein, presenilin 1, and presenilin 2, the majority of associated GWAS variants (~77%) are located in non-protein coding regions of the genome. While it is hypothesized that these variants and/or variants in linkage disequilibrium (LD) with them alter regulatory elements thereby changing gene expression, identifying the functional variant contributing to risk in noncoding regions is complex. First, the index variant from GWAS may not be functional. Rather any of the variants in LD with the index could be the molecular 'driving' variant. Second, regulatory regions can lie significant distances away from the genes that they modulate. Thus, the nearest gene to the index variant may not be the gene whose expression is modified. Lastly, chromatin structure and epigenetic marks are often tissue specific. As such, there remains a gap in knowledge regarding the molecular mechanisms altered by the AD associated variants.

Since characterization of 20 AD GWAS associated variants is outside the scope of this project, the focus of this project will be on variants in the PICALM genome locus. This locus has been replicated as a highly significant AD associated region, however, despite significant re-sequencing efforts, no coding or other functional variants have been identified. Therefore, this represents an excellent opportunity to identify functional regulatory variants alterations in this region, understand how they affect cellular biology, and establish a protocol to expand this to other GWAS loci.

Through a systematic, multidisciplinary genomic approach the research team will characterize variants in the PICALM locus to identify the underlying biology the associations represent by accomplishing the following aims: **1)** Develop a massive parallel reporter assay (MPRA) to identify effects of variation on gene expression. The researchers will first use publically available data on histone modifications and transcription factor binding sites in AD affected brain regions to prioritize regions of interest within the PICALM region. Then, they will directly query alternative haplotypes in those regions for effect on gene expression with an in vitro massively parallel reporter approach to identify the specific variant(s) in the region exerting regulatory capacity. **2)** Determine biological effects of candidate functional variants. The research team will further characterize regulatory variants in a cellular model approach using induced pluripotent stem cell (iPSC) derived neuronal cells (e.g. cortical neurons and/or microglia) modified to reflect the identified variant by genome- editing technology (e.g. CRISPR/Cas9). AD-specific (e.g. production of amyloid beta) and general cellular phenotypes (e.g. cell viability and lipid transport) will be evaluated. This proposal will help develop a streamlined protocol to study large noncoding regions in the context of AD. The identification of functional noncoding variants in AD can lead to novel entryways in treatment of AD delaying onset or progression of disease. The central aim of this project is to identify pathways involved early in neurodegeneration, increasing the impact of new treatment options through its potential application in multiple disorders.

26. **Grant #8AZ26:** Investigating The Role Of SORL1 In Alzheimer's Disease

Principal Investigator: Derek Dykxhoorn, PhD

Organization: University of Miami

Abstract of Proposed Research: The number of patients with neurodegenerative diseases is increasing significantly worldwide. Thus, research is ongoing to uncover mechanisms of disease to identify molecular targets for therapeutic intervention. Alzheimer's disease (AD) is the leading cause of dementia in the elderly and its prevalence continues to grow as the average lifespan increases. Genetic studies have identified a variety of genes and variants associated with AD. Although these findings have shed light on important aspects of Alzheimer's biology, many questions regarding the underlying mechanisms remain unknown. Evidence from our group and others demonstrate that the sortilin-related receptor 1 (SORL1) gene is a strong AD candidate gene. SORL1 has been implicated in both early and late onset AD. SORL1 is highly expressed in neurons in the brain, particularly in the hippocampus, Purkinje cells, and portions of the brain stem. The SORL1 gene encodes a protein involved in signal transduction that act in neurogenesis and neuronal maintenance. Studies have demonstrated that SORL1 interacts with the amyloid precursor protein (APP) and prevents it from entering the amyloidgenic pathway, as well as degrading nascent amyloid beta (Ab) peptides, thereby contributing to AD pathogenesis. In addition, SORL1 expression is reduced in AD susceptible regions of brains from AD patients. Thus, evidence suggests that SORL1 has the potential to act in a protective manner against Ab toxicity. The research team has previously identified a family with multiple affected individuals with single nucleotide deletion (g.4292delG) that causes a frameshift in SORL1 (p.Cys1431Ser*1). This is predicted to produce a truncated protein that lacks key functional domains. Therefore, SORL1 may play a protective role against AD and variants that reduce SORL1 expression or impair protein functionality may contribute to AD risk. However, additional studies are necessary to fully comprehend how SORL1 acts in AD pathogenesis.

To further elucidate the role of SORL1, the researchers propose the following two aims: 1) To characterize neurons from patient- derived induced pluripotent stem cell (iPSC) lines with the SORL1 alteration. They have already derived iPSC from two affected siblings bearing the p.Cys1431Ser*1 variant, and also have samples from two additional siblings bearing the mutation. These lines, as well as iPSC lines from ethnically matched non-demented aged individuals, will be differentiated into A) cortical neurons in 2D cultures and B) 3D cerebellar organoids. These 2D and 3D cultures will be functionally characterized for deficits in endosomal trafficking, cell viability, and morphological abnormalities in neurite outgrowth and branching as well as for AD-specific cellular phenotypes, including production of pathogenic Aβ and tau species. 2) Determine if the SORL1 p.Cys1431Ser*1 alteration is sufficient to drive AD pathogenesis utilizing genome editing. The iPSC lines generated in the first aim will be altered via CRISPR genome editing to correct the SORL1 variant in the cells derived from affected individuals and, conversely, introduce the SORL1 variant into the control iPSC lines. If correcting the SORL1 mutation can ameliorate the cellular and AD-specific phenotypes identified in aim 1, then this demonstrates causality. If introducing the alteration in control lines creates AD-specific phenotypes, this will show sufficiency.

27. **Grant #8AZ27:** Emerging Role Of Tau Citrullination In Alzheimer's Disease

Principal Investigator: Maj-Linda B. Selenica, PhD

Organization: University of South Florida

Abstract of Proposed Research: Alzheimer's disease (AD) is a devastating disease that impacts the life of over 5 million Americans living with the disease and their caregivers. Its pathology consists of amyloid beta and tau pathology, neuronal loss and ultimately cognitive decline. Brain inflammation is a crucial feature of AD pathogenesis. This study plans to describe a novel and irreversible post-translational modification of tau that has implication in its pathology and inflammation. To date, the researchers have demonstrated breakage of blood brain barrier (BBB) and infiltration of circulating immune cells in the brains of AD-like animal models, suggesting a role of these cells in neuroinflammation. BBB can recover integrity when tau levels are reduced and immunotherapy against tau reduces both pathology and neuroinflammation. Recently, the infiltration of a subset of circulating immune cells (neutrophils) has been shown in AD brains. Neutrophils are responsible of a phenomenon known as neutrophil extracellular traps (NETs), necessary for the bacterial entrapment and control of bacterial infections. NETs develop in animal AD models and individuals with AD, suggesting that these formations may induce neuroinflammatory processes and promote pathogenesis. Peptidylarginine deiminase (PAD) enzymes carries out citrullination of arginine residues by replacing the positive charge to neutral citrulline. This modification can have significant consequences on protein structure and function. Importantly, PAD4 is essential for the production of NETs.

The laboratory staff have identified a significant increase in protein levels of PAD2 and 4 in the brains of animal models of tauopathies, suggesting that tau pathology induce citrullination and NETs formation. They also have found increased PAD4 gene transcripts in the brains of AD compared to control patients. Importantly, the researchers are the first to identify several putative citrullinated sites on tau by PAD4, two of which confirmed with novel antibodies in animal models of tauopathy. Altogether it suggests that tau pathology induces PAD4 and in vivo citrullination of tau. They do not know how tau citrullination by PADs impacts its fate. The objective of this study is to first determine if PAD4 overexpression promotes tau citrullination and exacerbates tau aggregation in an animal model of tauopathy. To accomplish this, the researchers will induce the expression of PAD4 via adeno-associated virus and determine the extent of tau citrullination. They will also drug-induce PAD4 expression and NETs formation in the periphery of tau transgenic mice to determine if myeloid-induced PAD4 expression promotes tau citrullination in the brain. They will measure several components of the tau phenotype, previously established in their laboratory. Secondly, they will determine if immunization of transgenic mice against citrullination mitigates tau pathology, neuroinflammation and NETs formation. Since citrullination of tau has never been described before, the success of this study will provide new knowledge in the AD field. More importantly, it will provide novel tools and potential biomarkers for developing translational strategies with direct outcome on the pathogenesis of this devastating disease.

28. **Grant #8AZ28:** Microglial Phenotype In Alzheimer's Disease.

Principal Investigator: Kevin Nash, PhD

Organization: University of South Florida

Abstract of Proposed Research: The increasing need to identify novel therapies that halt or reverse the progression of Alzheimer's disease (AD) is urgent. Despite significant efforts there has been limited therapeutic success to date. However, a significant contributor to AD pathology progression, that has yet to be examined, is increased inflammation. It has been shown that increased inflammation contributes significantly to disease progression and that altering neuroinflammation may offer a novel therapeutic approach to AD. However, evidence-based knowledge of the inflammatory involvement in AD and other neurodegenerative diseases is relatively limited. The Fractalkine protein (FKN, CX3CL1) contributes to suppression of microglial activation and, in turn, decreased neurodegeneration in a number of neurological disorders, including AD. In AD, a large reduction in FKN has also been observed in postmortem cortical tissue. The researchers have been studying FKN and its contribution to neurodegeneration for a number of years and have demonstrated a beneficial effect of FKN agonism in multiple neurodegeneration models. They have shown that overexpression of a soluble form of FKN (sFKN) is capable of decreasing tau pathology in vivo in a tauopathy model, Tg4510. More important, the research staff have seen significant reductions in brain atrophy and neuron loss. Most recently, they have observed that increased FKN expression is able to reduce the deficits seen in these Tg4510 mice. The results from these observations have shown benefits in other neurodegenerative diseases such as Parkinson's disease (PD). In PD mouse models, MPTP toxicity, and -synuclein overexpression, the sFKN overexpression, but not a mutant FKN that is resistant to cleavage to become soluble, can reduce disease pathology and neuron loss. Positive activity with soluble versus membrane-bound FKN suggests that a soluble molecule has the potential to activate the receptor and achieve neuroprotective effects. Thus, FKN agonism is a potential therapeutic approach to treat AD.

Currently there are a very limited number of tools for examination of inflammatory involvement in neurological disorders. However, FKN offers a unique potential to further understand inflammation in neurodegenerative diseases such as AD and PD. Furthermore, since it is known that aging results in a primed microglial state, understanding the mechanism of microglial activation may have significant impact on the aged brain. In this proposal, the research staff will examine in detail, microglial activation in AD animal models and how this is altered with FKN agonism. Using Tg4510 mice (which express the human tau transgene) they will compare these transgenic mice to normal control mice and transgenic mice which over express FKN. Microglia will be isolated from each mouse group and a proteomic and RNA sequencing analysis will be performed. This comparison will extend current knowledge on how microglia may be contributing to AD progression and more importantly how altering the microglia can reduce AD pathology. Targeting the immune system offers a potentially novel therapeutic system that has yet to be explored for neurodegeneration and increasing understanding of its contribution to AD will be immensely valuable to the research community.

29. **Grant #8AZ29:** Divergent RanBP9 Signaling In Tau Pathogenesis

Principal Investigator: David E. Kang, PhD

Organization: University of South Florida

Abstract of Proposed Research: Tau pathology is common to multiple neurodegenerative diseases, including Alzheimer's disease (AD), Frontotemporal dementia (FTD), Progressive Supranuclear Palsy (PSP), Corticobasal degeneration (CBD), and others. However, AD is unique in that amyloid-beta (Abeta) accumulation is thought to be a principal driver of tau pathology. Despite the pivotal significance of Abeta in AD, multiple studies have also shown that Abeta-induced neurotoxic signals require tau, since the loss of tau abrogates many deleterious effects of Abeta. Thus, molecular intermediates of Abeta to tau signaling represent attractive molecular targets for therapeutic intervention. However, at present, a significant knowledge gap exists in terms of how Abeta pathogenically impinges on tau. Previous research findings indicate that the scaffolding protein RanBP9, which is highly elevated in brains of AD patients, functions as a molecular intermediate between Abeta to tau signaling and ultimately promotes tau pathology via two divergent pathways: 1) Hsp90/Hsc70-based preservation of tau; and 2) cofilin-induced dislodging of tau from microtubules. However, not enough evidence explains how RanBP9 alters the structure and aggregation of tau via its direct association with tau and/or Hsp90/Hsc70 complexes nor is there data indicating which activation state of cofilin promotes tau pathology in brain. In this proposal, the research team will seek to answer these important questions, which will further the understanding of Abeta-driven tau pathogenesis and aid in the development of potential therapeutics for AD.

To answer these questions, 1) The laboratory staff will utilize combinations of purified recombinant proteins (RanBP9, tau, Hsp90, & Hsc70) to determine how these protein interactions alter tau structure, assembly, and aggregation. They will also determine the role of RanBP9 complexes with Hsp90 and Hsc70 on tau aggregation and neurotoxicity in neurons. 2) The researchers also plan to directly test the hypothesis that activated cofilin but not inactive cofilin promotes tau pathology by expressing defined cofilin variants in brains of tau transgenic mice (tau pathology model) to assess synaptic function, microtubule integrity, and tau pathology.

30. **Grant #8AZ30:** Exploiting GPRC6a Antagonists To Mitigate Tau Deposition

    Principal Investigator: Daniel C. Lee, PhD

    Organization: University of South Florida

    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 facilitates transport of these drugs across the BBB would dramatically expand this research group's inventory of effective AD pharmaceuticals. This delivery technology will

also help 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 on 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 both the BBB and 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, which respectively 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 surface display's power as a technology platform for engineering proteins with binding properties, such as specific interaction with BBB endothelium relative to other endothelial cell types, well-suited to particular biomedical applications. During this project, the research team will utilize yeast display to isolate a collection of twenty-five to 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 virtue of each member Fn3 possessing unique biomolecule-binding properties. Using a brain-targeted, drug-carrying Fn3s will offer exciting potential to successfully make AD treatment and prevention program individualized.

31. **Grant #8AZ31: RELINQUISHED**

32. **Grant #8AZ32:** Longitudinal assessment of BDNF levels with Bacopa monnieri treatment in those at risk of developing Alzheimer's dementia

    Principal Investigator: Andrew Keegan, MD

    Organization: University of South Florida

    Abstract of Proposed Research: Alzheimer's disease (AD) is a neurodegenerative disease that progresses relentlessly, but it can have an insidious onset that early on is not always discernible from normal aging. Prior to overt, measurable memory deficits, individuals may display alterations in mood (e.g. depression, anxiety) or have subjective memory complaints. However, cognitive testing at this stage is often unremarkable (subjective cognitive decline). During this "preclinical" period and the critical years prior, there may be an opportunity to alter important circuitry in the brain in the regions of

memory consolidation (hippocampus) and emotion (amygdala) thereby reducing risk of progression to Alzheimer's dementia. These brain regions have been shown to be atrophied in persons with AD as well as those with depression without dementia. More importantly, and possibly prior to this atrophy, certain factors important to maintaining synaptic connections such as brain-derived neurotrophic factor (BDNF) may be reduced. Some studies have shown that low BDNF may put someone at greater risk of developing AD, and one large longitudinal study revealed that elevated BDNF may be protective from developing AD. Therefore, one approach of disease prevention may be to improve BDNF levels prior to and during the preclinical years. It is well known that important lifestyle choices such as exercise will reduce risk of developing AD and interestingly exercise has been associated with increasing BDNF levels (although there are likely many factors surrounding exercise benefits). It has also been shown that major stressful events may result in lowering BDNF. Additionally, there is evidence that commonly used anti-depressants including selective serotonin reuptake inhibitors (SSRI's) can raise BDNF in the treatment of depression but also alter amyloid levels in the spinal fluid both of which may be important for slowing the onset of AD. With these encouraging findings, there are plans to evaluate SSRIs as treatment as a preventative for AD, but there are other substances that can influence BDNF levels as well as amyloid processing. Bacopa monnieri is an Ayurvedic herbal that has been used for centuries for a variety of purposes including cognitive enhancement. Animal studies support multiple mechanisms of Bacopa including those important to AD: reducing amyloid pathology, increasing free radical scavenging, improving vascular flow, and increasing BDNF. In addition, the clinical data illustrates a good safety profile of Bacopa with multiple small studies showing efficacy in enhancing cognition and treating mood disorders. Our primary objective is to assess the effect of Bacopa on BDNF levels and other blood based biomarkers over time in an at-risk population for AD. Secondary objectives will include assessments of cognition and mood as well as safety labs. We will account for exercise and stress during this treatment period and incorporate other factors such as APOE and BDNF genetic carrier status. As clinical interventions move to alternative strategies beyond primarily modifying amyloid and/or tau, the focus on preserving or improving synaptic plasticity will likely be included. The above study may offer insight into some of these mechanisms while we wait for newer agents in phase 3 clinical trials for depression (ketamine related agents: esketamine, rapastinel) that may also offer hope in treating dementia due to potential synaptogenesis.

APPENDIX B

FISCAL YEAR 2017-2018 ACTIVE GRANTS

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
7AZ01	University of Florida	Smith, Glenn	$ 450,000	$ 150,000	$ 300,000	2/01/2017	3/31/2019	No	No	Yes
7AZ02	Florida International University	Burke, Shanna	$ 99,994	$ 33,330	$ 66,664	2/01/2017	3/31/2019	No	No	No
7AZ03	University of South Florida	Kang, David	$ 250,000	$ 83,334	$ 166,666	1/26/2017	3/31/2019	No	Yes	No
7AZ04	University of Miami	Rotundo, Richard	$ 250,000	$ 83,334	$ 166,666	2/01/2017	3/31/2021	No	No	No
7AZ05	Mayo Clinic Jacksonville	McLean, Pamela	$ 250,000	$ 83,334	$ 166,666	2/01/2017	3/31/2021	No	No	No
7AZ06	University of Florida	Bizon, Jennifer	$ 100,000	$ 33,333	$ 66,667	1/25/2017	3/31/2019	No	No	No
7AZ07	Mayo Clinic Jacksonville	Carrasquillo, Minerva	$ 250,000	$ 150,000	$ 100,000	3/14/2017	3/31/2021	No	No	No
7AZ08	Mayo Clinic Jacksonville	Dickson, Dennis	$ 100,000	$ 33,333	$ 66,667	2/01/2017	3/31/2019	No	No	Yes
7AZ09	University of Miami	Harvey, Philip	$ 86,792	$ 52,074	$ 40,726	2/27/2017	3/31/2019	No	No	No
7AZ10	University of Florida	Janus, Christopher	$ 100,000	$ 60,000	$ 40,000	2/14/2017	3/31/2019	No	Yes	No
7AZ11	University of Central Florida	Sugaya, Kiminobu	$ 100,000	$ 17,646	$ 82,354	2/06/2017	3/31/2021	No	No	No
7AZ12	University of Florida	Rincon-Limas, Diego	$ 249,998	$ 83,334	$ 166,664	2/06/2017	3/31/2021	No	No	No
7AZ13	University of South Florida	Gulick, Danielle	$ 100,000	$ 22,222	$ 77,778	2/14/2017	3/31/2019	No	No	No
7AZ14	University of Miami	Curiel, Rosie	$ 249,846	$ 83,283	$ 166,563	2/23/2017	3/31/2019	No	No	No

7AZ15	Mayo Clinic Jacksonville	Allen, Mariet	$ 100,000	$ 60,000	$ 40,000	2/06/2017	3/31/2019	No	No	No
7AZ16	Relinquished									
7AZ17	Mayo Clinic Jacksonville	Ertekin-Taner, Nilufer	$ 308,807	$ 68,624	$240,183	2/06/2017	3/31/2021	No	No	No
7AZ18	University of Miami	Loewenstein, David	$ 249,980	$ 83,328	$ 166,652	2/27/2017	3/31/2021	No	No	No
7AZ19	University of Florida	Levites, Yona	$ 250,000	$ 83,334	$ 166,666	2/01/2017	3/31/2019	Yes	Yes	No
7AZ20	University of Miami	Cukier, Holly	$ 250,000	$ 83,334	$ 166,666	2/01/2017	3/31/2021	No	No	No
7AZ21	Mayo Clinic Jacksonville	Cook, Casey	$ 250,000	$ 57,693	$ 192,307	2/01/2017	3/31/2021	No	No	No
7AZ22	Mayo Clinic Jacksonville	Kanekiyo, Takahisa	$ 250,000	$ 44,118	$ 205,882	2/01/2017	3/31/2021	No	Yes	No
7AZ23	University of South Florida	Cheng, Feng	$ 98,449	$ 32,814	$ 65,635	2/01/2017	3/31/2019	No	No	No
7AZ24	University of South Florida	Gamsby, Joshua	$ 100,000	$ 33,333	$ 66,667	2/01/2017	3/31/2019	No	Yes	No
7AZ25	University of Florida	Giasson, Benoit	$ 250,000	$ 93,750	$ 156,250	2/01/2017	3/31/2021	No	No	No
7AZ26	University of Miami	Wahlestedt, Claes	$ 100,000	$ 17,646	$ 82,354	2/01/2017	3/31/2021	No	No	No
7AZ27	University of Central Florida	Tatulian, Suren	$ 100,000	$ 23,076	$ 76,924	2/01/2017	3/31/2021	No	No	No
7AZ28	Florida Atlantic University	Wiese, Lisa	$ 93,133	$ 21,140	$ 71,993	5/15/2017	6/30/2019	No	No	No
6AZ01	Mayo Clinic Jacksonville	Murray, Melissa	$ 250,000	$ 232,143	$ 17,857	1/29/2016	1/31/2018	No	Yes	No
6AZ02	Florida Atlantic University	Galvin, James	$ 250,000	$ 230,357	$ 19,643	2/11/2016	1/31/2018	No	No	Yes
6AZ03	University of Miami	Loewenstein, David	$ 88,470	$ 82,151	$ 6,319	1/28/2016	1/31/2018	No	Yes	No

6AZ04	University of Miami	Czaja, Sara	$ 249,096	$ 231,303	$ 17,793	1/20/2016	1/31/2018	No	No	Yes
6AZ05	University of Florida	Cottler, Linda	$ 250,000	$ 232,143	$ 17,857	2/23/2016	1/31/2018	No	No	No
6AZ06	Mayo Clinic Jacksonville	Fryer, John	$ 250,000	$ 230,257	$ 19,643	1/29/2916	1/31/2018	No	Yes	No
6AZ07	University of Florida	Wicklund , Meredith	$ 250,000	$ 232,143	$ 17,857	2/23/2016	1/31/2018	No	No	No
6AZ08	University of Miami	Wahlestedt, Claes	$ 250,000	$ 232,143	$ 17,857	1/20/2016	1/31/2018	No	Yes	No
6AZ09	Florida State University	Terracciano, Antonio	$ 250,000	$ 232,143	$ 17,857	2/11/2016	1/31/2018	No	No	No
6AZ10	University of Florida	Horgas, Ann	$ 249,436	$ 231,619	$ 17,817	2/23/2016	1/31/2018	No	No	No
6AZ11	Florida State University	Meckes, David	$ 81,499	$ 75,678	$ 5,821	1/29/2016	1/31/2018	No	Yes	No
6AZ12	Mayo Clinic Jacksonville	Lucas, John	$ 200,000	$ 185,714	$ 14,286	2/11/2016	1/31/2018	No	No	Yes
6AZ13	Mayo Clinic Jacksonville	Kim, Jungsu	$ 200,000	$ 185,714	$ 14,286	1/29/2016	1/31/2018	No	No	No
6AZ14	University of West Florida	Guttman, Rodney	$ 81,499	$ 75,678	$ 5, 821	1/19/2016	1/31/2018	No	No	Yes
6AZ15	University of Florida	Bowers, Dawn	$ 100,000	$ 92,857	$ 7,143	2/11/2016	1/31/2018	No	Yes	No

ACTIVE RESEARCH GRANTS FISCAL YEAR 2017-18
(Funding Year 2016-2017)

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

Principal Investigator: Glenn Smith, PhD

Organization: University of Florida

Progress Report: In this project, a three-site consortium was 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. The research compares 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.

To date, researchers conducted the first intervention (8/7-8/18) with six couples and has screened perspective candidates enrolled five participants in the project. Five candidates have received MRI scans. Ongoing participation in this group has been nearly 100%. Mayo Jacksonville conducted its first session 10/9-10/20 with three couples all receiving MRI scans. Tallahassee Memorial Hospital (TMH) enrolled its first couple and is screening five more couples. They will conduct their first session 11/6-11/7.

Follow On Funding: Alzheimer's Association Clinical Fellowship, $150,000.00

Collaborations: Shellie-Anne Levy, UF post-doctoral fellow has received a Alzheimer's Association Clinical Fellowship to Promote Diversity award ($150,000 over 2.5 years) related to the PEACEOFMND study. A post-doctoral fellow is involved with the project at Mayo Jacksonville. TMH is recruiting Florida State University students to participate in delivery of the intervention at their site.

Journals: None at the time of reporting

Patents: None at the time of reporting

2. **Grant #7AZ02:** Demographic, Neuropsychological And Functional Classification, Risk Factors, And Progression Rates Of Individuals Diagnosed As "Impaired Not MCI" In The National Alzheimer's Coordinating Center Database Using Algorithmic Diagnosis

   Principal Investigator: Shanna L. Burke, PhD

   Organization: Florida International University

   Progress Report: Alzheimer's disease (AD) is a neurodegenerative brain disease which eventually manifests as clinically evident deficits in memory, thinking, and behavior. AD research indicates that there is a recognized preclinical phase between normal cognition and mild cognitive impairment (MCI) classified by some investigators as PreMCI. A somewhat equivalent state has been identified in the National Alzheimer's Coordinating Center Uniform Data Set classification, known as "impaired not-MCI (NotMCI)." The objectives for this study are threefold: 1) To subclassify NotMCI into well-defined entities, using a diagnostic algorithm, 2) To explore the demographic characteristics (age, sex, education, ethnicity, primary language), protective factors, and risk factors associated with subtypes of NotMCI, and 3) To explore rates of progression to MCI and dementia of subtypes of NotMCI. Thus far, the research project has been dedicated to determining formalized inputs to the algorithm and programming the algorithm to produce outputs with certain input cut-offs. By using a diagnostic algorithm and applying it to participants classified as NotMCI, the researchers aim to further define this cognitive status and its associated factors to improve the possibility of early intervention and possible disruption of the course of the disease should innovative treatments become available.

   Follow On Funding: None at the time of reporting

   Collaborations: The University of Florida, Florida International University, and Dr. David Loewenstein (from the University of Miami).

   Journals: None at the time of reporting

   Patents: None at the time of reporting

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

   Principal Investigator: David Kang, PhD

   Organization: University of South Florida

   Progress Report: The aim of this proposal is to further characterize the 'C' series of Slingshot inhibitor compounds and their analogs in multiple assays, including in vitro, cell-based, crystallographic, and medicinal chemistry. Based on prior cell-based studies, we have determined that the C2 compound is likely the most promising scaffold on which to place focus initially. However, we will assess the CI compound and its derivatives as well. A synthetic route has been developed that allows access to the scaffold of active compounds CI and C2. This route allowed for the synthesis of these compounds, which confirmed their activity in the p-Nitrophenyl Phosphate (pNPP) assay. In conjunction with the docking studies performed in the Chen laboratory, we have designed two different areas to approach with respect to lead optimization. The first of

these will allow us to determine if the portion of the molecule believed to reside outside of the active site binding pocket will allow us to introduce functionality that will improve the pharmacokinetic properties and, ideally, the blood brain barrier permeability. The latter will seek to replace the carboxylate portion with a phosphonate, which should serve as a better mimic of the phosphate substrate. More than ten different compounds based on C2 have now been synthesized. In regard to crystallography, researchers have been optimizing conditions to prepare complex crystals of Slingshot homolog 2 **(**SSH2) with novel inhibitors, particularly C2. Previous soaking experiments led to the discovery of phosphate molecules from the protein stock buffer residing in the protein active site and blocking the entry of inhibitors, as a result of strong hydrogen bonds between the phosphate and active site residues. To prevent phosphate from interfering with inhibitor binding for complex structure determination, we have tested and identified the minimal phosphate concentration needed in the protein stock buffer, as well as mutating the active site cysteine to alanine. This has allowed researchers to introduce a phosphotyrosine into the active site using soaking in a control experiment. The complex structure also demonstrates that the mutation did not alter the conformation of the phosphate moiety in the active site. It is being investigated whether crosslinking reagents such as glutaraldehyde can strengthen the SSH2 crystals, as previously done for other proteins. Further optimization of these conditions will allow us to stabilize the crystals during soaking and obtain high quality complex crystals. To determine whether the 'C' series of compounds show specificity for Slingshot, analysis is underway on the activity of these compounds on several other dual specific phosphatases (DUSPs), including Map kinase phosphatases (MKPs), tumor suppressor gene (PTEN), and phosphatases of regenerating liver (PRL). Preliminary studies indicate that both CI and C2 compounds, as well as several other analogs possess 3-20-fold higher inhibitory activity towards SSH1 versus PRL. We have now expressed PTEN in Sf9 insect cells to purify the recombinant protein ourselves, as we had done for SSH1. Now purified, the PTEN protein-from-Sf9 cells, and in vitro assays show activity. Preliminary studies indicate that the C1 and C2 compounds have three to ten times higher potency toward SSH1 than PTEN or PRL. Further studies are under way to test the activity, potency, and specificity of the ten new C2 derivatives.

Follow On Funding: None at the time of reporting

Collaborations: USF, two graduate students in training (performing cell-based studies and x-ray crystallography)

Journals:
Liu, T.f Wang, F., LePochat, P., Woo, J.AxTrotter, C., Kang, D.E*. (2017). Cofiiin mediated neuronal apoptosis via p53 translocation and PLD1 regulation. Scientific Reports. 14;7(1):11532. doi: 10.1038/s41598-017-09996-3. PMCID: PMC5599510

Woo, J.A., Liu, T., Trotter, C., Fang, C.C., De Narvaez, E., LePochat, P., Maslar, D., Bukhari, A., Zhao, X., Deonarine, A., Westerheide, S.D., Kang, D.E. (2017). Loss of function CHCHD10 mutations in cytoplasmic TDP-43 accumulation and synaptic integrity. Nature Communications 8: 15558.

Woo, J.A., Liu, T„Zhao, X., Trotter, C., Yrigoin, K., Cazzaro, S., De Narvaez, E., Khan, H., Witas, R., Bukhari, A., Makati, K., Wang, X., Dickey, C.A., Kang, D.E. (2017). Enhanced tau pathology via RanBP9 and Hsp90/Hsc70 chaperone complexes. Human

Molecular Genetics In press. ddx284,
10.1093/hmg/ddx284.

Patents: None at the time of reporting

4. **Grant #7AZ04:** Enhanced Acetylcholinesterase Expression Induced By Donepezil And Galantamine

   Principal Investigator: Richard L. Rotundo, PhD

   Organization: University of Miami

   Progress Report: 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 (AD). 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, the unpublished preliminary studies at this lab showed 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. 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. Thus, the purpose of this project is to clarify the molecular mechanisms of this unpredicted side effect of the two major drugs used for treating AD.

   During the first six months of the research project, the laboratory staff were able to determine which of the several different types AChE inhibitors enhanced AChE folding efficiency. The results showed that only the active site-directed inhibitors could enhance AChE folding. Based on this data, the research team will continue to evaluate combinations of inhibitors for their effects on AChE activity and their ability to reduce the enhanced folding effect. The knowledge gained for this study could 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.

   Follow On Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals: None at the time of reporting

   Patents: None at the time of reporting

5. **Grant #7AZ05:** How Does Alpha-Synuclein Contribute To Tau Dysfunction In Alzheimer's Disease?

Principal Investigator: Pamela McLean, PhD

Organization: Mayo Clinic Jacksonville

Progress Report: The focus of this project is to determine if there are previously undetected forms of alpha-synuclein (asyn) and tau in Alzheimer's disease postmortem brains that could contribute to the disease. To accomplish this goal, the research team used neurodegenerative model systems to probe a role for tau-asyn interactions in the progression of Alzheimer's disease and other dementias. Currently, progress towards this overall goal are proceeding as expected. In the first six months, they have optimized their detection assay to detect small soluble oligomers of alpha-synuclein in human brain tissue. While the optimization of the "in-house" proximity ligation assay (PLA) was successfully completed, the PLA assay did not work well on the cells in culture; resulting in the need to optimize the commercial DuoLink assay to facilitate the goals of this study. Using the brightfield DuoLink assay, the researchers have successfully demonstrated the asyn pathology can be detected using PLA in post-mortem brain tissue containing Lewy body from dementia patients. Using the PLA signal, the research team was able to confirm detection of the asyn and oligomeric asyn pathology. Presently the lab is in the process of using post-mortem brain samples from Alzheimer disease patients with varying tau agents to determine if small, soluble asyn oligomers can be detected after performing PLA. While Dr. SeHee Oh has replaced Dr. Jae-Hyeon Park as the post-doctoral fellow on this project, the research team expects no delays and plans to complete their specific aims on time.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

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

Principal Investigator: Jennifer Bizon, PhD

Organization: University of Florida

Progress Report: Intracellular inclusions comprised of tau proteins are among the earliest pathological features of Alzheimer's disease (AD), the most common age-associated neurodegenerative condition. Tau inclusions can emerge in the transentorhinal subregion of perirhinal cortex (PER) by the fourth decade of life, which is more than ten years prior to the average age of AD diagnosis. The consequences of this early tau pathology on cognition and disease progression, however, remain poorly elucidated. Existing mouse models have established critical links between tau pathology and medial temporal lobe circuit dysfunction, but current transgenic models lack the

anatomical specificity needed to recapitulate the earliest pathology in humans. Modeling tau pathology in aging rats offers advantages over mice, particularly for facilitating the preclinical evaluation of mechanisms that are most relevant to cognitive deficits in humans. Specifically, there is a rich literature describing cognitive changes in aged rats that can be leveraged to better elucidate the specific aspects of cognitive decline that signal transition to disease. By using this rat model, the research team can accomplish the long-term goal of this project; which is to develop sensitive cognitive assays for humans that provide a reliable index of early AD pathology that can be used for early intervention to improve long-term cognitive outcomes in affected individuals.

Considerable progress has been made on both aims of this project in the past year. Specifically, for Aim 1, human wildtype tau and a control construct has been successfully delivered to PER of both young and aged rats. Brains from these animals are now being evaluated for tau and synaptic integrity 2- and 6- month after delivery. Aim 2, is focused on determining the cognitive effects associated with tau pathology in PER. Two manuscripts have recently been published from the principal investigator laboratories describing perceptual discrimination deficits in aged rats on tasks of novel design that allow for sensitive gradients of discrimination ability to be measured in both visual and olfactory domains (Johnson et al., 2017, Hippocampus and Yodor et al., 2017, Neurobiology of Aging). Moreover, in the past year, the researchers have developed an automated version of the visual perceptual discrimination task that will aid in efficient longitudinal testing. A cohort of young and aged rats has now completed baseline assessment on these discrimination procedures and will soon undergo surgery for delivery of human wildtype tau to PER. Rats will be retested to assess perceptual discrimination ability at two and six month post-surgical timepoints to determine explicitly how tau pathology interacts with aging to impair cognition. These experiments should provide the foundation for using perceptual discrimination testing as a method for early detection of tau pathology and vulnerability to AD. Notably, the automated methods for measuring both olfactory and visual discrimination abilities developed in the principal investigator's laboratories have strong translation potential to humans.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

7. **Grant #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

Progress Report: 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 project is to improve the understanding of the influence of vascular disease risk factors and inflammation on AD in this minority population. Specifically, this study 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.

To date, the researchers have conducted a pilot experiment for the measurement of plasma RNA levels. The plasma RNA was extracted and transcript levels were measured using a pre-developed Nanostring "codeset" that contains probes to measure the expression level of 760 genes, that have previously reported to be implicated in neurodegenerative diseases. From the data collected, the amount of plasma was finite, and did not increase the input of RNA, as was expected. As a result, the researchers will experiment with an increased cycle number for the pre-amplification process to determine if increasing the pre-amplification cycle number can enhance transcript detection. If this experiment is successful, the knowledge gained from this study could lead to new and more relevant treatments, and ultimately preventive therapies.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

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

Progress Report: 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.

Currently in this research study, almost half of the cohort has been completed. The research team has searched 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. After processing over nine hundred slides, the remaining cases and slides from the tissue samples will be prepared and immunostained for processing in the following months. Medical records from athletic participants has been collected, and additional record review is ongoing. The data extracted from the individuals include disease course (onset and duration), family history, education, substance use (alcohol, tobacco, and caffeine), head trauma, height and weight, and any neurological or neuropsychological testing score. The long-term goal is that after comparing these findings, important insight toward understanding the pathophysiology of TBI and its contribution to AD progression will become known.

Follow On Funding: 2017-2018 Younkin Scholars Award (Synaptic Biology Program, at Mayo Clinic), $32,500.00

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

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

    Principal Investigator: Philip Harvey, 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). Early diagnosis helps pave the way for increasingly more targeted treatment interventions. This research fellowship grant is focused on training post-doctoral research student, Dr. Daema Piña to study more innovative ways of assessing and diagnosing persons in the earliest stages of neurodegenerative diseases such as Alzheimer's disease (AD). The mentorship team, which includes Dr. Harvey (who serves as the primary mentor), Dr. David Loewenstein and Dr. Rosie Curiel, assist in providing training experience in the R01 project at the University of Miami and the Alzheimer's Disease Research Center (ADAC) project at Mount Sinai Medical Center. Along with working on the abovementioned projects, Dr. Piña has continued to receive specialized training in preclinical AD through her work on two funded aging and cognition studies at the University of Miami Center on Aging. She also evaluates patients with

neurodegenerative illness (primarily Mild Cognitive Impairment and AD) at the State of Florida funded Memory Disorders Clinic. In addition, Dr. Piña has also been involved with co-authoring two manuscripts and has participated in the preparation of two grants; one Ed and Ethel Moore grant and an NIA RO1 grant submitted recently in October 2017. During the remainder of this fellowship training program, Dr. Piña will be directly involved in collecting data on the longitudinal R01 study. She will also receive training in the analysis and conceptualization of longitudinal data which includes neuropsychological and neuroimaging components.

Follow On Funding: None at the time of reporting

Collaborations: Based on her training history, the postdoctoral fellow re-established a relationship with the neuropsychology team at Jackson Memorial Hospital to share in didactic training and offered to give a presentation on Alzheimer's disease to their attendings, fellows, and doctoral students on November 17, 2017.

Journals: None at the time of reporting

Patents: None at the time of reporting

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

    Principal Investigator: Christopher Janus, PhD

    Organization: University of Florida

    Progress Report: 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. The pivotal research aim of the grant is to develop anti-Corticotropin-Releasing Hormone (CRH) antibodies and establish their efficacy in reducing CRH levels in a brain. To this end, within the second quarter of the grant duration, the research study determined dissociation constants (Kd) of anti-CRH antibodies, evaluated the ability of these antibodies to block acute stress-induced increases in corticosterone, and created single-chain variable fragment (scFv) antibody constructs from anti-CRH antibodies. These constructs have the ability to be packaged into recombinant adeno associated viral vectors and expressed directly within the brain, bypassing blood brain barrier. The research team has implemented the anti CRH-active vaccination and rAAV transduced Anti CRH scFv's in mouse models

relevant to Alzheimer's Disease (AD) to assess their efficacy in blocking acute stress induced increases in corticosterone, A3 levels, and tau phosphorylation. Mice have been immunized with novel anti-CRH epitopes in order to generate a second round of anti-CRH antibodies that may have higher affinity to CRH. A novel ovalbumin (OVA) conjugated immunogen, that induced anti CRH titers in in all immunized mice (n = 7), has been identified. This immunogen is currently being utilized as an active vaccine in AD-like mouse models of amyloidosis and will have results over the next few months. In addition, a novel method to affinity mature our scFv constructs that will allow us to use an error Prone PCR protocol, is being developed. This error prone PCR method will take the current scFv constructs and introduce a few mutations per copy of DNA. Through generating a library of millions of mutant scFvs, the team will evaluate and purify very high affinity scFv's that will be able to be used to accomplish the experimental aims.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals:
Futch. H.S.. Croft, C.L., Truong, V.Q., Krause, E.G., Golde, T.E.Targeting psychologic stress signaling pathways in Alzheimer's disease. Molecular Neurodegeneration. 12(1):49, (2017)

Patents: None at the time of reporting

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

Progress Report: Alzheimer's disease (AD) 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. 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 AD. 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 therapeutic antibody against IL1RAP will be attached to the exosomes using click chemistry. AD patient derived induced pluripotent stem cells (iPS cells) will be used to study the effect of these new therapeutic delivery systems. AD 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 AD 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. Currently, researchers are developing exosomes carrying IL1RAP antibodies and study their blocking effects in AD patient derived iPS cells. In order study all these, first step is to develop exosomes carrying the antibodies. As of the current reporting, researchers successfully developed exosomes collection methods and exosome protein loading vectors, where RFP protein were loaded into the exosomes. They are working on adding IL1RAP antibodies to the exosome and import to the neural cells derived from the patient's iPS cells. However, the efficacy has not reach to the expected level and conditions are being optimized.

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 #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

Progress Report: 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. 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.

USDA permits have been applied for. Researchers began working on the logistics of the project and have designed a plan of action. A postdoctoral candidate has been identified for this project. The list of 6,484 fly strains was classified based on molecular function, which will facilitate the analysis and interpretation of the genetic screen. Reagents have been acquired to further characterize the Abeta+tau expression in the research model, which is currently underway.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

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

Principal Investigator: Danielle Gulick, PhD

Organization: University of South Florida

Progress Report: Alzheimer's disease (AD) 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. Many patients with AD also struggle with sundowning syndrome (SS), 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. With the aim that SS can be treated with drugs that will reset the internal clock, the research team used two mouse models of SS to test whether treatment with an inhibitor of casein kinase 1, a key enzyme in the circadian clock, is sufficient to reduce the symptoms of sundowning by improving cognition, socialization, and reducing anxiety.

Thus far, the researchers have built up their aged C57BL/6J and APP-PS1 mouse colonies, so that they have sufficient mice to test their two drug doses in both the circadian chambers and in the behavioral assays. They have completed the behavioral assays in C57BL/6J mice and are currently about half way through scoring and analyzing their data. They have also run the first two cohorts of C57BL/6J mice in the circadian chambers and are about to begin the third cohort. Recently the lab has received an institutional equipment award for a Lumicycle, which will allow them to add a new molecular analysis, assessing changes in the molecular circadian rhythm in real time following their drug treatment. The research team has also received an additional grant to cover the costs of their circadian housing for an additional year, allowing them to run the studies constantly, without sharing the space with other researchers at the institution. Now that the aged mouse colony is old enough and large enough for more robust drug studies, the research team plans to test their drug treatment on the APP/PSI mice and run molecular analysis on tissue samples previously collected. Furthermore, they plan to complete all behavioral drug studies and analysis, and finish the molecular analysis of their cohorts during the duration of their grant period.

Follow On Funding: None at the time of reporting

Collaborations: We are now collaborating with Dr. Jim Leahy in the USF Department of Chemistry to test novel investigational compounds that may reset the clock and improve cognition with greater specificity than PF-670462

Journals: None at the time of reporting

Patents: None at the time of reporting

14. **Grant #7AZ14:** A Consortium To Study Precision-based Computerized Assessment For The Detection Of Mild Cognitive Impairment In Older Adults

   Principal Investigator: Rosie E. Curiel, PhD

   Organization: University of Miami School of Medicine

   Progress Report: With the rapidly aging population, early detection of cognitive decline in individuals at risk for Alzheimer's disease (AD) is a global priority. 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. With a focus to be at the forefront of this critical area, this proposed project plans to administer three novel computerized tests to 120 older adults (40 normal elderly, 40 amnestic mild cognitive impairment: [aMCI] and 40 Preclinical AD participants). The objective is that half of these subjects will be primary Spanish-speakers while half will be primary English speakers. Currently the research team has piloted twenty individuals to make modifications in both languages. They have carefully collaborated with the Department of Computer Sciences regarding which equipment is best to run programs and interface with the participants. The laboratory staff are in the initial phase of the recruiting process and will be ready to enroll participants in the weeks ahead. The research team has been trained on the protocol and proper conduct of the instruments, and have already compiled a list of potential enrollees for the study.

   Follow On Funding: None at the time of reporting

   Collaborations: Collaborations have been established with the University of Miami's Center for Computer Sciences which will develop and further refine the computerized instruments under investigation. One fulltime doctoral level computer science student, Lloyd Beaufils has been working on the development of the tests under the supervision of the Graduate Student Director and Assistant Professor, Ubbo Visser, PhD.

   Journals: None at the time of reporting

   Patents: None at the time of reporting

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

   Principal Investigator: Mariet Allen, PhD

Organization: Mayo Clinic Jacksonville

Progress Report: 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. Under Aim 1, the researchers proposed to investigate three genetic loci previously implicated in risk for Alzheimer's disease, and for which evidence suggests a role in altered expression of these genes in disease pathophysiology. The minimum capture region of gene +/- 10kb, together with linkage disequilibrium (LD) information, target regions (and co-ordinates) for each locus was determined. The design was finalized and has been approved by the principal investigator (PI) for manufacture, and reagents ordered. They are well positioned to rapidly apply these customized procedures for alignment, data quality control, and variant filtering upon completion of sequencing for the design.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

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

    Principal Investigator: Nilufer Ertekin-Taner, MD, PhD

    Organization: Mayo Clinic Jacksonville

    Progress Report: 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). 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 is 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. The data that was generated under the prior Florida Department of 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. This project's aims are to: 1) Expand the cohort of additional samples; 2) Launch studies of gene expression pathways utilizing blood RNA samples; and 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. The collaborating institutions Mount Sinai Miami Medical Center (Dr. Maria Greig-Custo is site principal investigator (PI)) and University of Florida (Dr. Meredith Wicklund is site PI) have approved IRB. The PI met with the study coordinators and provided sample and data collection materials. An inventory was completed of the African-American subjects in our database and updated diagnoses and IRB information where applicable. Sample collections at Mayo Clinic Florida is ongoing under existing protocols. This project was delayed due to Hurricane Irma.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Collaborations: University of Florida (Dr. Meredith Wicklund is site PI) and Mount Sinai Miami Medical Center (Dr. Maria Greig-Custo is site PI).

Journals: None at the time of reporting

Patents: None at the time of reporting

17. **Grant #7AZ18:** Brain Amyloid Load And Novel Cognitive Measures In Diverse Ethnic Groups

Principal Investigator: David Loewenstein, PhD

Organization: University of Miami School of Medicine

Progress Report: Early detection of Alzheimer's disease (AD) is extremely important since novel and emerging treatments are likely to be effective in the earliest stages of the disease before multi-system brain degeneration has occurred. Amyloid positron emission tomography (PET) scanning has emerged as one of the most sensitive methods to detect early AD pathology but is extremely expensive and not available in most settings. After having developed novel cognitive measures in White Non-Hispanic groups with high rates of amyloid deposition in the brain, it was unknown as to whether these novel cognitive tests relate to amyloid in the same way among different ethnic and cultural groups in Florida. To observe this scientific question, the researchers examined 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 (African-American and Hispanic). The data collected from this examination was essential in establishing the utility of novel cognitive stress tests in epidemiological and clinical studies.

In the first several months of the study, the researchers contracted with Piramal Imaging and set the PET scan protocol in the Department of Nuclear Medicine. Despite disruptions from a hurricane that delayed initial set-up, the staff have recruited 18 individuals who have obtained full Florbetaben amyloid scans and have tentatively scheduled fifteen more participants for the study. Thus far, all amyloid PET scans have been successfully run and analyzed by a neuradiologist and will be subject to quantitative SUVR analyses in the future. All scientific aspects of this new project are going smoothly. While there is not yet sufficient data to publish the research findings, this exciting study may help improve the identification of early Alzheimer's disease in different minority populations that will be critical with emerging treatments. With the aid of a federal grant awarded to Dr. Loewenstein, additional data was collected on these subjects via neuropsychological stress tests, structural MRI and blood genotyping. The goal over the next three years of this project is to obtain amyloid PET scans minority African-American and Hispanic older individuals who are at higher risk for Alzheimer's Disease.

Follow On Funding: None at the time of reporting

Collaborations: Principal Investigator Dr. David Loewenstein and Co-Investigator Dr. Rosie Curiel at the University of Miami Miller School of Medicine collaborated with Piramal Imaging and the Department of Nuclear Medicine to set up PET scan protocols.

Journals: None at the time of reporting

Patents: None at the time of reporting

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

Principal Investigator: Yona Levites, PhD

Organization: University of Florida

Progress Report: Anti-tau immunotherapy has recently emerged as a promising approach to target tau, found in Alzheimer's disease (AD), but many mechanistic questions regarding the optimal form of anti-tau immunotherapy remain open. The hypothesis of this project is that anti-tau immunotherapy may be optimized by targeting both intracellular and extracellular pools of tau and that specific binding of hyperphosphorylated Tau by single chain variable fragments (scFv) or by intracellularly expressed intrabodies will prevent its toxicity and formation of neurofibrillary tangles, or even solubilize existing Tau. To test this hypothesis, the research team has cloned and expressed six functional antibodies, fused to Trim21, in cell culture Tau aggregation model and tested whether they prevent aggregation of phosphorylated Tau. At this point they have confirmed expression and are in the process of analyzing the data from cell culture. In the months to come, they will clone these functional antibodies into the pAAV vector in order to package the virus for animal injections. To begin this next

phase, they have established two in vitro models to screen novel antibodies and scFvs and started evaluation of fast in vivo Tau aggregation model.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals:
Generation and Characterization of New Monoclonal Antibodies Targeting the PHF1 and AT8 epitopes on human Tau Kevin Strang; Marshall Goodwin; Cara Riffa; Brenda Moore; Paramita Chakrabarty; Yona Levites; Todd Golde; Benoit I Giasson , Acta Neuropathologica Communications, in press.

Patents:
The University of Florida filed two patents based on this research project.
UF#16782 Disclosure entitled "New Phospho-Independent tau Antibody 2D1"
UF#16620 Disclosure entitled "New Phospho-tau Antibody 7F2"

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

Principal Investigator: Holly N. Cukier, PhD

Organization: University of Miami

Progress Report: Alzheimer's disease (AD) is the leading cause of dementia among the elderly. While over 20 genetic loci have been identified, genes are still being revealed that contribute to AD risk. The research team recently identified a p.S1038C change in the TTC3 gene that segregates with the disease in 11 individuals in an extended family. To understand the role that TTC3 and the alteration could play in AD, the focus of this study is to examine the consequence of the p.S1038C change on the function of neurons derived from induced pluripotent stem cells (iPSCs). During the first few months of the grant, it was believed that a new IRB approval would be required for this project but after verifying that the project did not meet the federal definition of research requiring a new IRB review, the study was able to proceed as normal. The researchers began ordering the supplies necessary to begin this study and planned out the first experiments. During the initial experimental phase, two of the three iPSC lines did not grow well after prolonged incubation in liquid nitrogen (~three years) and as a result had to be reprogrammed from peripheral blood mononuclear cells (PBMCs). Since then, the research team has completed the reprogramming of these cells, and validated that all three iPSC lines carried the p.S1038C alteration. These initial results were presented in a poster at the 67th Annual Meeting of the American Society of Human Genetics in Orlando, Florida, from October 17-21, 2017. Currently, the CRISPR/Cas9 genome editing method is being optimized and experiments are ongoing to determine that it is successful in HEK293 cells prior to attempting in stem cells, which will be more difficult.

Due to Hurricane Irma making landfall in Florida in September of 2017, the University of Miami Miller School of Medicine was closed from September 7th-12th. To prepare for this natural disaster, all tissue culture experiments had to be halted in advance of this emergency closing and cells frozen in liquid nitrogen. Following the storm, experiments

were resumed as normal. The aim for the next twelve months is to complete the optimization of the CRISPR procedure for the TTC3 p.S1038C variant and to perform it on each of the three AD patient-derived stem cell lines to correct the alteration as well as the reciprocal experiment on three control iPSC that are derived from aged, neurologically normal individuals to introduce the alteration. These lines will then be thoroughly assessed to determine if there are any molecular, cellular, or morphological features that are distinct between cases and controls, and if these features are ameliorated with genome editing, thereby demonstrating causality of the TTC3 variant of interest. Upon successfully completion of these aims, the researchers plan to complete RNA-seq experiments from both the iPSC-derived neurons and the brain samples, and complete the final analysis and verification of the top hits of interest.

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

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

    Principal Investigator: Casey Cook, PhD

    Organization: Mayo Clinic Jacksonville

    Progress Report: The overall goal of the current project is to investigate the mechanism(s) by which the A152T tau variant increases risk of Alzheimer's disease (AD). A significant scientific accomplishment during the reporting period includes the finding that tau mRNA levels are elevated in AD patients carrying the A152T variant, which may indicate that the A152T mutation impacts AD risk by stabilizing tau mRNA species and increasing expression. In addition, to explore whether altered phosphorylation patterns on tau might contribute to increased AD risk conferred by the A152T mutation, several AAV constructs were generated and produced, and subsequently expressed in vivo. Therefore, an additional scientific accomplishment of the current reporting period was the finding that preventing phosphorylation on the neighboring T153 residue in the presence of the A152T mutation inhibits both loss of brain weight and hyperphosphorylation on S202 (CP13 epitope), which are key features of the A152T-AAV model. Both of these key findings are being confirmed in either a larger cohort of animals, or in patients carrying the A152T variant and diagnosed with non-AD tauopathies. There are no significant changes in key personnel, scientific programs, shared resources and/or institutional commitments that have impacted this research project.

    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

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

    Principal Investigator: Takahisa Kanekiyo, MD, PhD

    Organization: Mayo Clinic Jacksonville

    Progress Report: Population-based epidemiologic studies have revealed that Alzheimer's disease (AD) and vascular cognitive impairment and dementia (VCID) are major causes of dementia in the aged population. Therefore, the overall goal of this project is to determine how cerebrovascular dysregulation during aging contributes to the pathogenic pathways for AD using in vitro and in vivo models. In addition, because the ε4 allele of the apolipoprotein E (APOE) gene increases the risk for VCID and AD among its three polymorphic alleles (ε2, ε3 and ε4), the research team will also study apoE-isoform-dependent mechanisms in the pathway. During the reporting period, the significant progress toward these goals have been observed. In particular, it was found that brain pericytes, the mural cells of the capillary walls, differentially modulated endothelial cell phenotypes in an apoE isoform-dependent manner. Extracellular matrix (ECM) protein induction, tubular structure formation, and barrier formation were lower with endothelial cells co-cultured with pericytes isolated from apoE4-targeted replacement (apoE4-TR) mice compared to those from apoE3-TR mice. Importantly, the apoE4-TR mice had decreased ECM protein expression and increased plasma protein leakages compared to apoE3-TR mice. These results identified apoE in brain pericytes as a critical regulator of endothelial function in an apoE isoform-dependent manner, potentially contributing to the risky effect associated with apoE4 in AD and aging-related cognitive decline. In the following years, the researchers will continue to further assess how vascular aging contributes to the apoE isoform-dependent mechanisms.

    Follow On Funding: None at the time of reporting

    Collaborations: None at the time of reporting

    Journals:
    Yamazaki, Y., Kanekiyo, T. (2017). Blood-Brain Barrier Dysfunction and the Pathogenesis of Alzheimer's Disease. International Journal of Molecular Science. 18(9):1965.

    Patents: None at the time of reporting

22. **Grant #7AZ23:** System Analysis Of Potential Drug Interactions In The Treatment Of Alzheimer's Disease From The FDA Reporting System, Electronic Health Records And Protein Interaction Networks

    Principal Investigator: Feng Cheng, PhD

    Organization: University of South Florida

Progress Report: Recently clinical studies showed that the Alzheimer's disease (AD) patients are at an increased risk of drug-drug interactions (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. 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. This research will develop and evaluate 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 more appropriately. Thus far, adverse drug event reports have been imported into a relational dababase. The PharmacoVigilance Signal Detection system was installed to analyze FAERS data. The human protetin-protein interaction (PPI) network has been downloaded and imported into a database so that researchers can develop a random-walk-based algorithm to identify DDI-related proteins. ADEs for dementia patients on cognitive supporting therapy were gathered. Patients were assessed based upon diagnosis and a medication regimen was administered based on a Dementia Rating Scale-2, Geriatric Depression Scale, and Mini-Mental Status exam, and presence of cognitive impairing medications. Information was collected to assess quality life indicators such as incidence of falls and hospitalizations. Currently the researchers are assessing the data to determine if there was an increase in adverse drug reactions in those patients because of polypharmacy.

Follow On Funding: None at the time of reporting

Collaborations: Two PharmD students have been enrolled to check the drug adverse events and drug prescription from the electronic health records of the Byrd Alzheimer's Institute.

Journals: None at the time of reporting

Patents: None at the time of reporting

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

    Principal Investigator: Joshua Gamsby, PhD

    Organization: University of South Florida

    Progress Report: Circadian rhythm sleep disorder (CRSD) is a common, yet poorly understood symptom of neurodegenerative diseases, such as Alzheimer's disease (AD).

Presently, there is no effective treatment for CRSD in AD, largely because it is unclear how the circadian clock is impacted by AD neuropathology. Most of the work directed at elucidating the link between CRSD and AD has focused on mouse models of β-amyloid pathology, thus it is unclear how tauopathy contributes to the disruption. Preliminary data generated from using a transgenic mouse model of tauopathy (Tg4510) showed that these mice displayed increased bouts of wakefulness during the light cycle (when they should be sleeping) and were arrhythmic in constant darkness at an age when tauopathy was present. The research team also found a disruption of the molecular clock in both the hypothalamus, the site of the master clock, and in the hippocampus in aged Tg4510 mice. Aim 1 is focused on extending these findings by assessing the functional status of the circadian clock in aged Tg4510 mice. The hypothesis to be tested is that tauopathy, like β-amyloid pathology, impairs normal circadian rhythmicity of the sleep/wake cycle. The status of the molecular clock will be analyzed in aged Tg4510 mice by comparing the variation in levels of core clock protein levels in the hypothalamus and hippocampus of non-transgenic and transgenic mice over the course of a day. Since receiving the research grant, significant progress has been made in achieving the aims outlined in the original proposal. This progress was made even though there was a significant setback in that the collaborator for this research, who was providing the mouse line required for this study (the Tg4510 line), moved to another institution which required the researchers to generate their own colony. The most significant accomplishments achieved over this reporting period were as follows: A Tg4510 colony was established; it has been determined that tauopathy disrupts the circadian clock at a very early age (~two months); and a novel technique has been established to determine the impact of tauopathy on the molecular circadian clock in live cells. In addition to this scientific progress, the research staff have published these preliminary findings in the Experimental Neurology journal. Dr. Gamsby has also presented these findings at the Circadian Clocks Symposium: Time of our Lives at Dartmouth College.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

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

Patents: None at the time of reporting

24. **Grant #7AZ25:** Understanding The Molecular Mechanisms Of Seeding And Transmission Of Wild Type And Mutant Tau

Principal Investigator: Benoit Giasson, PhD

Organization: University of Florida

Progress Report: The accumulation of 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, this study will explore the relative effects of wild-type and additional disease-associated mutants in cellular and animal models. Intriguingly, preliminary data generated in the 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. All studies described in the research aims are ongoing. The research team is in the process of completing most of the studies using HEK293T cells proposed in the research plan. Researchers are completing the characterization and analyses with our new monoclonal antibody to tau phosphorylated at Ser305. Most of the somatic brain transgenesis studies are ongoing and are now analyzing many of the cohorts of mice.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

25. **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

Progress Report: Currently approved treatments for Alzheimer's disease (AD) are not efficacious and are palliative in some patients at best. There is an enormous need for new drugs. One of the drugs that has showed promise to date is resveratrol (RSV). This research project will study a new oral formulation of RSV – JOTROL – that has been developed by a Florida-based company (Jupiter Orphan Therapeutics) and shows markedly higher bioavailability when compared to unformulated resveratrol. 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 SIRT1 in brain, which is likely to be more pronounced by equimolar doses of JOTROL. The 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 has been assembled with vast experience in Alzheimer's disease, resveratrol chemistry and epigenetics. Research staff are optimizing JOTROL doses for treatment of animals. Because Alzheimer's disease is largely age-dependent, a cohort of transgenic Alzheimer's

disease mouse models (animals engineered to express three human genes linked to Alzheimer's) is currently aging to proceed with the aims. Current results suggest that some genes reported to be protective for Alzheimer's disease are affected by JOTROL.

Follow On Funding: None at the time of reporting

Collaborations: The principal investigator (PI), a staff scientist and graduate student at the University of Miami Miller School of Medicine have been involved with this research project. JOTROL compound has been obtained from the Florida-based company, Jupiter Orphan Therapeutics.

Journals: None at the time of reporting

Patents: None at the time of reporting

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

Principal Investigator: Suren A. Tatulian, PhD

Organization: University of Central Florida

Progress Report: Despite extensive basic and clinical research, no effective therapies have been developed for Alzheimer's disease (AD). Diagnostic, passive and active immunotherapy trials have been directed to tau protein and certain amyloid beta (AB) species, such as AB42 and AB40. Pyroglutamylated AB (ABpE) occurs in AD brains at 10-50% of total AB and is hypertoxic. Yet, little research has been conducted towards characterization of structure and cytotoxicity of ABpE alone and especially in combination with unmodified AB. Thus, the molecular composition and structural features of "toxic AB oligomers" have remained elusive. The data indicate that, contrary to current paradigm that ABpE promotes fibrillogenesis and thereby exerts hypertoxic effect, AB and ABpE reciprocally inhibit intermolecular B-sheet formation and shift the aggregation process towards hetero-oligomers of intramolecular H- bonding. Moreover, AB/ABpE hetero-oligomers elicit unsurpassed cytotoxicity as compared to oligomers of individual peptides or fibrils. This project addresses Priority/Focus Area 2.4 "Novel Biomarkers," and aims at detailed characterization of the structure and cytotoxicity of AB/ABpE hetero-oligomers as potential new AD biomarkers by pursuing the research aims. Aggregation and accompanying structural changes of AB_{1-40} and AB_{pE3-40} have been studied by thioflavin -T fluorescence and circular dichroism spectroscopy. Membrane pore formation by AB_{25-35} has been studied. Atomic force microscopy images have been obtained on AB_{1-42} and AB_{pE3-42} peptides and their combinations incubated in aquepus buffer for 24 hours. Efforts have been made to induce neuronal differentiation of PC12 cells using collagen and nerve growth factor.

Follow On Funding: None at the time of reporting

Collaborations: Four graduate students (Nabin Kandel, Faisal Abedin, Molla Manjurul Islam, Leslie Davis) and one undergraduate student (Abdel Naser) have been trained/mentored during the reporting period. A manuscript has been submitted for publication, with Nabin Kandel (PhD student) as first author.

Journals: None at the time of reporting

Patents: None at the time of reporting

27. **Grant #7AZ28:** Dementia Detection And Treatment Benefits Through Home Health Care As Compared To Clinic Care In A Rural Florida Underserved Population

    Principal Investigator: Lisa Kirk Wiese, PhD

    Organization: Florida Atlantic University

    Progress Report: A culturally diverse (56% African-American, 5% Afro-Caribbean, and 34% Hispanic) and underserved rural region in southcentral Florida referred to as "the Glades" faces higher risks of Alzheimer's disease (AD) and work AD-associated health outcomes due to multiple health disparities. There is a higher percentage of residents over age 65 (26.5%) compared nationally (18%), more than 30% live below the poverty level, and only 65% received a high school diploma. The Glades is a designated Medically Underserved and Health Professional Shortage Area. The project hypothesis is "Compared to current practice, offering health care access to a nurse practitioner at a local Florida clinic site or home health visit through a "ROAD-NEXT-Steps" approach will be more effective in early detection of Alzheimer's disease, as evidenced by follow up cognitive evaluations after screening." "ROAD" is the acronym for "Rural Older Adults with Dementia." The study has four specific aims that have been implemented. Aim 1: Determine patterns of dementia-specific assessment, diagnosis, and treatment as a function of age, race, ethnicity, gender, education, and health literacy. Aim 2: Describe differences in rates of diagnosis and treatment between at-home versus clinic based assessments. Aim 3: Evaluate differences in patient preferences between at-home versus clinic-based assessments. Aim 4: Pilot-test a "ROAD-Next-Steps" referral program after cognitive assessment in a community of rural Florida, ethnically diverse, older adults. Health care professionals and health educators for the project have been hired and trained, including cultural training. Education and cognitive screening events for the Glades have been implemented.

    Follow On Funding: None at the time of reporting

    Collaborations: Florida Atlantic University Nurses Christian Fellowship chapter

    Journals: None at the time of reporting

    Patents: None at the time of reporting

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: Two major studies, which are currently in manuscript preparation, are directly related to having received State of Florida funding from the Ed and Ethel Moore Alzheimer's Disease Research program. The first study has identified intriguing clinicopathologic differences between autopsied Hispanic Americans and European Americans, along with exploratory findings in a smaller African American cohort. Frequency of neurodegenerative diseases is examined in the overall cohort, but the most significant findings were identified in the autopsy-confirmed AD cohorts.
The second major study investigated sex differences in autopsy-confirmed AD. Through this study, it was found that in the State of Florida brain bank, the overall frequency of autopsy-confirmed AD did not differ between women and men. This finding was observed in Hispanic Americans, African Americans, and European Americans. The research team is currently preparing a manuscript that reveals the clinical impact of this finding. From the inception of the grant, the team did not experience change in scientific programs, shared resources and/or institutional commitments that impacted research. Although there was no change in key personnel, the technician dedicated to the genetic portion of the grant went on maternity leave for four months. This has delayed progress with respect to obtaining genetic data.

Follow On Funding: None at the time of reporting

Collaborations: The research team is collaborating with Otto Pedraza, who is working to develop a community-based program for Hispanic Americans in Duval County.

Journals:
Shinohara M., Murray M.E., Frank R.D., Shinohara M., DeTure M., Yamazaki Y., Tachibana M., Atagi Y., Davis M.D., Liu C.C., Zhao N., Painter M.M., Petersen R.C., Fryer J.D., Crook J.E., Dickson D.W., Bu G., KanekiyoT. (2016) Impact of sex and APOE4 on cerebral amyloid angiopathy in Alzheimer's disease. Acta Neuropathologica 132(2):225-34. PMID: 27179972

Graff-Radford J., Lesnick T.G., Boeve B.F., Przybelski S.A., Jones D.T., Senjem M.C., Gunter J.L., Sarro L., Ferman T.J., Knopman D.S. Murray M.E., Dickson D.W., Jack Jr. C.R., Petersen R.C., Kantarci K. (2016) Predicting survival in Dementia with Lewy Bodies with hippocampal volumetry. Movement Disorders 31(7):989-94. PMID: 27214825

Lowe V., Curran G., Fang P., Liesinger A.M., Josephs K.A., Parisi J.E., Kantarci K., Boeve B.F., Pandey M.K., Bruinsma T., Knopman D.S., Jones D.T., Petrucelli L., Cook C.N., Graff-Radford N.R., Dickson D.W., Petersen R.C., Jack Jr. C.R., Murray M.E. (2016) An autoradiographic evaluation of AV-1451 Tau PET in dementia. Acta Neuropathologica Communications 4(1):58. PMID: 27296779

Tacik P., Contreras M.S., DeTure M., Murray M.E., Rademakers R., Ross O.A., Wszolek Z.K.,Dickson D.W. (2016) Clinicopathologic heterogeneity in FTDP-17 due to MAPT p.P301L mutation including a patient with globular glial tauopathy. Neuropathology and Applied Neurobiology (Epub ahead of print). PMID: 27859539

Raman M.R., Kantarci K., Murray M.E., Jack Jr. C.R., Vemuri P. (2016) Imaging Markers of Cerebrovascular Pathologies: Pathophysiology, Clinical Presentation, and Risk Factors. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 18(5):5-14. PMID: 28054023

McKeith I.G., Boeve B.F., Dickson D.W., Halliday G., Taylor J.P., Weintraub D., Aarsland D., Galvin J., Attems J., Ballard C., Bayston A., Beach T.G., Blanc F., Bohnen N.I., Bonanni L., Bras J.M., Brundin P., Burn D.J., Chen-Plotkin A., Duda J.F., El-Agnaf O.M.A., Feldman H., Ferman T.J., Ffytche D., Fujishiro H., Galasko D., Goldman J.G., Gomperts S.N., Graff-Radford N.R., Honig L.S., Iranzo A., Kantarci K., Kaufer D.I., Kukull W.A., Lee V.M.Y., Leverenz J.B., Lewis S., Lippa C., Lunde A., Masellis M., Masliah E., McLean P., Mollenhauer B., Montine T., Carretero E.M., Mori E., Murray M., O'Brien J.T., Orimo S., Postuma R., Ramaswamy S., Ross O.A., Salmon D.P., Singleton A.B., Taylor A., Thomas A., Tiraboschi P., Toledo J.B., Trojanowski J.Q., Tsuang D., Walker Z., Yamada M., and Kosaka K. (2017) Diagnosis and management of dementia with Lewy bodies Fourth consensus report of the DLB consortium. Neurology 89(1):88-100. PMID: 28592453

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: Upon notification of funding, the research team began to meet regularly to develop the curriculum, training guide, pre- and post-test components, and revise the planned outcomes. This process continued over the first six months of the project. During this time, names of potential individuals, who would be interested in participating in the project, were collected. The research team revised and refined all materials and utilized the recruitment coordinator to engage community centers, senior housing, and local organizations to provide advanced notice of the start of the project. In addition, some formative pilot testing of the materials was initiated during this delay.

The team reconsidered the original research design, after losing a community center partner, to a quasi-experimental design with serial pre- and post-testing to examine gains in knowledge, mastery, self-efficacy, and care confidence. The team took advantage of this period by adding additional components gleaned from our formative work with caregivers and community leaders to include modules with mindfulness and health coaching. Mindfulness includes mediation, yoga, personal wellness, biofeedback and reflection as methods to reduce life stressors. The original module on diet and

nutrition was expanded, using guidance from the integrative nutritionist to expand the health coaching, lifestyle and attitude component of the project.

A challenge addressed was that although there was great interest from spousal caregivers to participate in the program, they were limited in their ability because of the need to provide supervised care for the patients. The team redesigned the intervention to account for the need to provide supervised time for the person with AD while the caregiver participated in the sessions.

While the training program was proceeding, the team's social worker recognized that providing caregiver training and coping skills would not, by itself, alleviate some of the psychological stress the caregivers were facing without some form of therapeutic counseling. The program was adapted once more to include those components and add a social work intern (MSW student) to the team to assist the licensed clinical social worker with initial evaluations and development of an individualized plan. The team engaged a family foundation which provided additional funding that allows the project to proceed after this grant ends. The Harry T. Mangurian Foundation provided a grant of $100,000 which permits an additional year of data collection and the development of enduring materials for the C4U program. This additional funding is critical to support the adaptive changes in the original design and make the program more tailored to the individual caregiver and their unique and individualized needs. The study team was able to adapt, reconfigure, revise, and substantially improved upon the original design into a personalized program that is increasingly likely to lead to improved caregiver outcomes and better patient care.

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: This grant award provided funding to support a post-doctoral fellowship in neuropsychology. In the role of post-doctoral fellow, Dr. Ailyn Penate trained in research methodology and statistics which is run by Drs. Loewenstein, Curiel, Crocco and Czaja at the University of Miami. Throughout the fellowship, she has authored two abstracts that have been accepted by the National Academy of Neuropsychology and the International Neuropsychology Society (INS). She has reviewed neuroimaging with Drs. Loewenstein and Duara and learned the MRI visual rating system of the hippocampus, entorhinal cortex and perirhinal cortex. Dr. Penate maintained the SPSS database for ongoing projects and helped Dr. Loewenstein entered data to assist with quality assurance. She oversaw maintaining this database

and is learning basic and more advanced statistical analysis of the data by Dr. Loewenstein and Curiel. Because of the outstanding work on the Ed and Ethel Moore post-doctoral fellowship, she was offered the position of a prestigious 1Florida ADRC scholar position.

Follow On Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals:
Crocco, E., Loewenstein, DA., Curiel, R., Alperin, N., Czaja, S., Harvey, PD., Sun, X., Lenchus, J., Raffo, A., Penate, A., Melo, J., Sang, L., Valdivia. R., Cardenas, K (2017). A Novel Cognitive Assessment Paradigm to Detect Pre-Mild Cognitive Impairment (PreMCI) and the Relationship to Biological Markers of Alzheimer's disease. Journal of Psychiatric Research. Aug 24; 96:33-38. doi: 10.1016/j.jpsychires.2017.08.015.

Loewenstein, DA, Curiel, RE, DeKosky, S., Roselli, M., Bauer, R., Grieg-Custo, M., Penate, A., Li, Chunfei, Lizagarra, G., Golde, T., Adjouadi, M. and Duara, R. (2017). Recovery from proactive semantic interference and MRI volume: A replication and extension study. Journal of Alzheimer's Disease.

Loewenstein, DA, Curiel, RE, Wright, C, Sun, X., Alpern, N, Crocco, E, Czaja, S, Raffo, A, Penate, A, Mello, J., Capp, K, Gamez, M and Duara, R (2017). Recovery from Proactive Semantic Interference in MCI and Normal Aging: Relationship to Atrophy in Brain Regions Vulnerable to Alzheimer's disease. Journal of Alzheimer's Disease. 56:3 1119-1126.

Dr. Penate has also just submitted a first authored paper comparing two novel cognitive stress tests which is under review by the Journal of Clinical Neuropsychology.

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: The research team is fortunate to not have experienced any challenges, delays or issues during this reporting period and have made significant progress on the project. Technology pertinent to the delivery of the program has been identified, acquired and programmed. In addition, the research team obtained approval from the University of Miami Institutional Research Board (IRB) approval to conduct of the study. The program content has been refined and finalized in English and Spanish for both study conditions. The research team is currently pilot testing the program and have begun recruitment effort in the community. The outreach effort was completed with the Community Advisory Board. This effort was recently expanded to include a luncheon with the pastors of the churches affiliated with the African American community, to raise awareness of Alzheimer's Disease in this community. As part of the study, an

Organization Planning Tool was also created to serve as memory and an information organizational aid for the care recipient. A library of virtual tours of Perez Art Museum in Miami, Fairchild Tropical Garden, and Miami Frost Science Museum, and Classical Music presentation was also created so they can be shared between the CG & CR as part of our pleasant events module. An overview of the project was presented as part of an invited symposium at the Annual Meeting of the Gerontological Society of America, July 2017, San Francisco.

Notably, the research team received a grant from the National Institutes of Health (5R01AG054009) to expand this project. It is funded from 09/01/2016 – 04/30/2021. With the additional funding from the NIH, an African American, Doris Caldwell was hired to join the study. Doris was brought on to help with recruitment of African American caregivers and the delivery of the program. Ideally, the NIH grant is providing the additional resources needed to expand the recruitment of the project to a large sample of White/Caucasian, Hispanic and African American dyads.

Follow On Funding: National Institute on Aging, $3,262,583

Collaborations: None at the time of reporting

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: Currently, 999 Florida residents have been interviewed and added to the Florida Registry of Older Adults for Research, and 179 residents have been screened on the Montreal Cognitive Assessment (MoCA) during this reporting period. About 10% scored in a range that necessitated a visit to a Florida Memory Disorder Clinic (MDC); however, few could be seen because the MDC requires a physician referral, which these people did not have. While it is believed that physician education is a necessary next step, a solution was provided for this barrier: the creation of a new referral plan with referral back to primary care practices rather than referral to a MDC. Thus, all participants scoring below the norm on the MoCA are now referred using this two-step process. The research team continues to build the Registry and the statewide infrastructure to link older adults to memory screening and related health research through procedural development, volunteer recruitment, expanding network partnerships and training for cognitive impairment screening. To build a more robust workforce throughout Florida capable of conducting this research, the study has added significantly to the online workforce development initiative. An online distance training procedure was constructed for new Community Health Workers (CHWs) and volunteers allowing our program to reach all parts of the state. A scheduling tracker has been created to track CHWs' and volunteers' outreach in their communities. Volunteer recruiting continues to present a challenge, but progress is being made in building the volunteer base. A MoCA

memory screening training was conducted for HealthStreet CHWs and volunteers and certified four CHWs to perform the MoCA on community members 65 years of age and older. Volunteers and non-UF students obtained approval to interact with and enroll study participants and view protected health information. The Alzheimer's Disease questionnaire included in the health needs assessment received IRB approval and has been administered on community members 65 years of age and older.

Follow On Funding: None at the time of reporting

Collaborations: Gulf Coast State College in Bay, Washington and Gulf Counties; Florida Keys Community College in Monroe County; Hodges University and Florida Gulf Coast University in Collier County; Seminole State College in Seminole County; Bethune-Cookman University and Stetson University in Volusia County; Chipola College in Jackson and Calhoun County; Florida Southwestern College in Hendry College; and Tallahassee Community College in Liberty County. In addition to educational institutions, there has been recent interest and discussion with Dr. Glenn Smith, University of Florida's Chair of the Department of Clinical and Health Psychology, regarding a future partnership with the HABIT® Healthy Action to Benefit Independence & Thinking program in Miami.

Journals: None at the time of reporting

Patents: None at the time of reporting

6. **Grant #6AZ06:** Clusterin Prevention Of Alzheimer Disease 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. The amyloid precursor protein (APP);CLU-/- mice show 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 were 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 ~four – fold increase in the amount of CAA, they have significantly less hemorrhage. This work has now been published in Proceedings of the National Academy of Sciences. The researchers will focus their attention on further mechanistic insights into CLU in CAA as well as the role of CLU in pathology.

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

Loss of clusterin shifts amyloid deposition to the cerebrovasculature via disruption of perivascular drainage pathways. Aleksandra M. Wojtas, Silvia S. Kang, Benjamin M. Olley, Maureen Gatherer, Mitsuru Shinohara, Patricia A. Lozano, Chia-Chen Liu, Aishe Kurti, Kelsey E. Baker, Dennis W. Dickson, Mei Yue, Leonard Petrucelli, Guojun Bu, Roxana O. Carare, and John D. Fryer PNAS 2017 114 (33) E6962-E6971; published ahead of print July 12, 2017, doi:10.1073/pnas.1701137114

Patents: None at the time of reporting

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

   Principal Investigator: Meredith Wicklund, MD

   Organization: University of Florida

   Progress Report: Through previously funded research (Duara et al, 2010), the ability to obtain a consensus diagnosis was determined to be more efficient without compromising integrity when utilizing an algorithmic method; this diagnosis is called AlgDx. The AlgDx is an algorithm to determine cognitive state of a patient using the physician diagnosis (PhDx) and neuropsychological diagnosis (NPDx). The novelness of the AlgDx is that it helps to avoid individual bias and saves time among multisite studies of MCI and dementia. Through this grant mechanism, the research team was able to build a computerized algorithm where the PhDx and NPDx can be extracted through databanks and calculate the AlgDx. In addition, the calculator has been embedded in the website for easy use, validation, and implantation among all consortium researchers. The goal is to validate this method, determine its efficiency and broadly disseminate for clinical use, if applicable. In addition, subjects have been recruited across the cognitive spectrum to undergo novel neuropsychological measures via standardized administration. The data has been combined with subjects recruited at Mount Sinai Medical Center in Miami through the 1Florida ADRC. The goal is to determine if these neuropsychological measures are more sensitive than traditional cognitive assessments in detecting early cognitive changes in Alzheimer's disease. Preliminary analysis of the data indicates that the Object Recognition Discrimination Task (ORDT) elucidates earlier cognitive decline than traditional memory measures, including Logical Memory and HVLT-R. In addition, analysis found that the pre-exposure trials of ORDT impairs performance of Cognitively Normal participants, because familiarity to the non-target object interferes with recognition of the target object. In contrast, pre-exposure improved the performance of the aMCI and Dementia groups, possibly because familiarity with the non-target was not strong enough to result in false recognition or interference.

   Follow On Funding: None at the time of reporting

Collaborations: This research led to interaction with HealthStreet, a community engagement program at the University of Florida that seeks out community members interested in referrals for research programs.

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: The study has progressed with the aims of the grant as planned. For Aim 1, Experiments have been completed and resulted in a publication. Clinical analogs of CTI-309 showed positive anti-Alzheimer effects on gene and protein expression of Alzheimer's disease biomarkers. Compound M344 was advanced for further testing. For Aim 2, compounds that were generated by our medicinal chemists were tested in the study's assays. Animal work progressed well and has mostly been completed. In addition, behavior data has been collected. Brain data looks promising and are still being evaluated. So far, the results indicate that inhibiting HDAC 3 has a strong effect on AD-related genes and memory. Compound CTI-350 reverses Alzheimer's disease (AD)-related memory impairment in the aged triple transgenic AD mouse model, increases expression of the neuroprotective protein BDNF and decreases tau protein phosphorylation at residue Ser202 (a hallmark of Alzheimer's disease). The research team is further analyzing different brain regions with Westerns, ELISAs and RT-PCR, with currently available data. The increased performance in Y-maze, Barnes maze, novel object recognition tests and nesting behavior, decreased plasma beta-amyloid (Aβ), tau phosphorylation and BACE levels show significant effects for normalizing Alzheimer's-like phenotype with no negative effects on other organs tested.

   Follow On Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals:
   Volmar C-H, Salah-Uddin H, Janczura K, Halley P, Lambert G, Wodrich A, Manoah S, Patel N, Sartor GC, Mehta N, Miles NTH, Dorcius D, Cameron MD, Brothers SP, Wahlestedt C (2017) M344 promotes nonamyloidogenic amyloid precursor protein processing while normalizing Alzheimer's disease genes and improving memory. Proc Natl Acad Sci In press, Early Edition: available at www.pnas.org/cgi/doi/10.1073/pnas.1707544114.

   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 study aims to test the hypothesis that Powerful Tools for Caregivers intervention will improve caregivers' outcomes (reduce burden and depressive symptoms, and improve self-care, physical activity, and quality of life) and reduce burdensome behaviors in care recipients with Alzheimer's disease and related dementias. During the reporting period, the research team has worked with community partners to advertise the study to reach the recruitment goals. The study was advertised through media outlets, with flyers at multiple community locations and events, and presentations at caregiver meetings. Additionally, participants have been continuously enrolled and tested for the study. Despite the challenges of recruiting caregivers in research studies, the team has surpassed the goal of 60 caregivers enrolled in the study, and we have reached 72 caregivers enrolled in the study. Recruitment continues to account for participants' dropout (n=9), which has been about 20%, and to account for participants interested in the study but currently unable to enroll in the classes. Indeed, while caregivers have been enrolled, many of them have difficulty finding the time to participate in the Powerful Tools for Caregivers and the study assessments. By Q3 2017, 59 participants have completed at least part of the study assessments, and more are completing the study. The research project is still ongoing and continues to be well received by the participating caregivers. The research team continues to have regularly scheduled and as-needed meetings to ensure the study's progression.

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 eight 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. The researchers are continuing to actively recruit participants for this study as recruitment remains a significant challenge. Thus far, they have screened (1) medical records, (2) clinic schedules, (3) conducted information sessions, (4) added new recruitment sites, and (5) consulted with recruitment experts. To date, they have enrolled four participants. Medical records in the University of Florida (UF) Health database have been screened to determine if potential participants meet the inclusion/exclusion criteria.

Since May 2017, the researchers have screened the clinic appointment schedules for geriatric and neurology clinics affiliated with UF Health. The researchers have also conducted several presentations at retirement communities and dementia daycare facilities in order to enroll more study participants.

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 Alzheimer's disease (AD) patients. The collection of brains and blood from our preclinical mouse model of AD for exosome purification was completed. Six brains (three Wild Type and three Alzheimer's disease) were harvested and up to 4mL of plasma from two, four, six, eight, and ten+ 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. Most recently, we validated potential biomarkers identified by mass spectrometry that are present in brain-derived exosomes from the preclinical mouse models of AD. Currently the researchers are determining the levels of proteins in exosomes isolated from younger animals to determine whether they are also early predictors of disease. Results obtained and confirmed in the mice will be tested in patient samples. They have determined the amount of plasma to obtain enough exosomal proteins and nucleic acids for downstream analyses and will continue the purifications and proceed with the RNA-Seq experiments described in the grant.

   Follow On Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals:
   Hurwitz, SN, Olcese, JM, Meckes DG, Jr. 2017. An optimized method for purification of whole brain-derived extracellular vesicles reveals insight into neurodegenerative processes Ininin mouse model of Alzheimer's disease.
   Submitted to Journal of Alzheimer's Disease.

   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 Jacksonville

    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, two of the three consortium sites have enrolled an overall sample of 386 participants (86% of the target sample). Of those currently enrolled, 116 self-identify as African American (non-Hispanic), 110 as Caucasian (non-Hispanic), and 160 Hispanic. The third consortium site (University of South Florida) was not able to initiate the study and enroll participants until October 2017. The data sharing agreement between Mayo Clinic Florida and the University of Florida Alzheimer's Disease Research Center was completed and they have created a merged dataset containing study data from the Mayo Clinic and Mt. Sinai Medical Center sites. In the coming months, the researchers plan to add data from the USF participants.

    Follow On Funding: National Institute on Aging, $2,538,663

    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 Jacksonville 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 Jacksonville

    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. During the last three months, the researchers screened 135 small molecule candidates for their effect on Apolipoprotein E using ELISA method. They identified several compounds that modulate Apolipoprotein E levels. Previously, small molecule candidates based on pharmacophore approach were tested in human ApoE3 astrocyte cells. Using the ApoE ELISA based screening method, they screened small organic molecules. The researchers plan to submit a grant with National Institute of Health (NIH) after completing our identification and optimization of lead compounds.

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 addresses 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 greatly increased by 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, elution is more effective. Improved elution of phage was also observed.

Progress has also been made in developing a robust qPCR approach. 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. Currently, it has been determined that our phage have better or equivalent sensitivity as the Tau-5 antibody in our assay. It was determined that substitution of our phage into the standard InnoTest kit, the phage did not perform as well. Based on these observations, researchers are working to develop a new version of the kit using phage only and attempt to screen our library against the sandwich-ELISA system that could be a direct substitute for InnoTest. Either of these results would be significant as the cost to utilize a phage-based system is fractional compared to an antibody-based system. Experiments continue to show promise as originally proposed and will result in a manuscript submission. Analyzed data will be developed into a manuscript showing that phage derived from this experimental approach can be excellent and low-cost substitutes for antibody-based assays for tau. The assay being

developed by the research team, if successful, would be expected to be a fraction of current costs.

Follow On Funding: University of West Florida, $9,000

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 Adenosine triphosphate (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 with additional participants. Additional potential participants, who have expressed interest, have been secured, and participant recruitment will continue.

    Currently we have 15 participants who have completed the two-week intervention, including eight who have undergone pre-post fmri and spectroscopy imaging. The second task has focused on analyses and interpretation of the neuroimaging data, particularly the spectroscopy data. When looking at effect sizes, researchers find rather potent intervention effects across a variety of metabolic factors derived from the 31P coil. The effect sizes range from mild to large. In the frontal region, the largest changes are seen in gamma and beta ATP, ATP/pi, polymerase chain reaction (PCR)/partial differential equation (PDE), PCR/PME, pH and magnesium. Fewer changes are observed in the temporal region, but include gamma-ATP, ATP/pi, PCR/pectin methylesterase (PME), PME/partial differential equation (PDE), and magnesium. An extensive literature reviews is being conducted to develop a better appreciation and understanding of the meaning of these changes.

    Follow On Funding: None at the time of reporting

Collaborations: Preeti Sinha, MD, a geriatric psychiatrist, from the National Institute of Mental Health and Neuroscience (NIMHANS) of Bangalore, India, has joined the team. Dr. Sinha is a Fogarty Fellow, through a grant that is directed by Dr. Cotter (principal investigator). 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 is entitled "Dose Response relationship between Near Infrared (NIR) Light Stimulation and Functional Brain Activity" (Institutional Review Board #2016014040).

Gene Alexander, Ph.D. from the University of Arizona. Based on preliminary analysis of the MRS-spectroscopy data, a small pilot grant was submitted to the McKnight Inter-Institutional Cognitive Aging and Memory Intervention Core for funding and is pending. This is a multisite proposal involving investigators at University of Florida (Bowers/Woods) and University of Arizona (Alexander) who are affiliated with the McKnight Foundation. The newly submitted project will extend the aims of the current grant by studying healthy older adults with normal age-related cognitive changes and by carrying out more extensive neuroimaging.

Journals:
Trifilio,E., Butterfield,L ,Mangal,P. Maye,J.,Marsiske, M.,& Bowers, D. (2017,February/ A Tale of Two Stories: Comparing Logical memory and Newcomer Stories

Sinha, P., Wood, A., Bowers, D. (in review, 2017). Is dose-response study the way to success in near infrared brain stimulation. (Photo Medicine and Laser Surgery).

Patents: None at the time of reporting

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