Sample Application for Small Business Funding

Through the Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) programs, NIA aims to help small businesses develop effective treatments and interventions for healthy aging. NIH small business funding is competitive, and resubmissions are a common and important part of the award process.

Find more NIA sample applications and information about SBIR/STTR funding:
https://www.nia.nih.gov/research/sbir/nia-small-business-sample-applications

PI: **kozikowski, alan P.**	Title: Study of the New HDAC6i SW-100 as a Treatment for Alzheimer's Disease and Other Tauopathies	
Received: 03/31/2017	FOA: PAS17-065	Council: 08/2017
Competition ID: FORMS-D	FOA Title: Advancing Research on Alzheimer's Disease (AD) and Alzheimer's-Disease-Related Dementias (ADRD) (R41/R42)	
1 R41 AG058283-01	Dual:	Accession Number: 4037760
IPF: 10045114	Organization: STARWISE THERAPEUTICS, LLC	
Former Number:	Department:	
IRG/SRG: ZRG1 ETTN-A (11)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted]	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: Early Stage Investigator:

Senior/Key Personnel:	*Organization:*	*Role Category:*
Alan Kozikowski	StarWise Therapeutics LLC	PD/PI
Marcia Gordon	University of South Florida	MPI
David Morgan	University of South Florida	MPI

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ❍ Application ● Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number [redacted]

5. APPLICANT INFORMATION **Organizational DUNS*:** [redacted]

Legal Name*: StarWise Therapeutics LLC
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: Dr. First Name*: Alan Middle Name: Paul Last Name*: Kozikowski Suffix:
Position/Title: CEO
Street1*: [redacted]
Street2:
City*: Chicago
County: Cook
State*: IL: Illinois
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 60614-4002
Phone Number*: [redacted] Fax Number: Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* R: Small Business

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍ Yes ● No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Study of the New HDAC6i SW-100 as a Treatment for Alzheimer's Disease and Other Tauopathies

12. PROPOSED PROJECT
Start Date* Ending Date*
01/01/2018 12/31/2018

13. CONGRESSIONAL DISTRICTS OF APPLICANT
IL-005

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Alan Middle Name: Paul Last Name*: Kozikowski Suffix:
Position/Title: CEO
Organization Name*: StarWise Therapeutics LLC
Department:
Division:
Street1*: [redacted]
Street2:
City*: Chicago
County: Cook
State*: IL: Illinois
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email* [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* [redacted]
b. Total Non-Federal Funds*
c. Total Federal & Non-Federal Funds* [redacted]
d. Estimated Program Income*

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ○ PROGRAM IS NOT COVERED BY E.O. 12372; OR

● PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Dr. First Name*: Alan Middle Name: Paul Last Name*: Kozikowski Suffix:
Position/Title*: CEO
Organization Name*: StarWise Therapeutics LLC
Department:
Division:
Street1*: [redacted]
Street2:
City*: Chicago
County: Cook
State*: IL: Illinois
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

Signature of Authorized Representative*
Alan P Kozikowski

Date Signed*
03/31/2017

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:1245-Cover Letter.3.11.17.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: StarWise Therapeutics LLC
Duns Number: [redacted]
[redacted]
Street2:
City*: Chicago
County: Cook
State*: IL: Illinois
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: IL-005

Project/Performance Site Location 1

❍I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: University of South Florida
DUNS Number: [redacted]
Street1*: [redacted]
Street2:
City*: Tampa
County: Hillsborough
State*: FL: Florida
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: FL-015

Additional Location(s) File Name:

Funding Opportunity Number: PAS-17-065. Received Date: 2017-03-31T12:25:30.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: __ 1 __ 2 __ 3 __ 4 __ 5 __ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number A4100-01

3. Is proprietary/privileged information included in the application?* ● **Yes** ❍ **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	1239-abstract.final.pdf
8. Project Narrative*	1240-narrative AD.pdf
9. Bibliography & References Cited	1241-REFERENCES.pdf
10.Facilities & Other Resources	1242-Resources.combined.pdf
11.Equipment	1243-Equipment.combined.pdf
12. Other Attachments	1244-SBC_001242051.pdf

Funding Opportunity Number: PAS-17-065. Received Date: 2017-03-31T12:25:30.000-04:00

ABSTRACT

Alzheimer's and other tauopathies are medical problems growing to historical proportions that threaten the long term viability of medical care systems world-wide. Alzheimer's costs today are 1.2% of the US GDP, and growing as the population ages. Effective disease-modifying treatments have yet to be developed. A number of drugs in clinical testing target amyloid, but very few have been developed to target tau. We expect that like heart disease, cancer and HIV, effective Alzheimer's management will require combination treatment using multiple therapeutic modalities. Prior work by our research team has determined that part of the tau phenotype can be reduced using a histone deacetylase 6 (HDAC6) inhibitor, Tubastatin A (TA). This involved treatment of Tg4510 mice that develop tau deposits by 3 mo and forebrain atrophy by 6 mo of age. We treated mice from 5 to 7 mo and found improved behavioral performance and reduced total tau deposition. However, other components of the tau phenotype in this model were not significantly impacted. Here we propose to test whether an improved HDAC6 inhibitor, **SW-100**, can more completely rescue the tau phenotype in this mouse. **SW-100** has a higher affinity, slightly longer half-life and substantially increased brain permeability than TA. SW-100 is a new HDAC6 inhibitor with selectivity similar to that of TA, but increased CNS penetration. **SW-100** further lacks mutagenicity in the Ames test (in which TA was positive). Thus, we wish to evaluate if this compound, as well as a newly designed back-up analog, can more fully reverse the phenotype of the Tg4510 mouse by pursuing the three aims below.
Aim 1. Prepare 4 new analogs of **SW-100** as potential back-up compounds, and conduct HDAC isozyme testing, tubulin acetylation assays, and ADMET assays. Advance the best of these to animal studies in Aim 2.
Aim 2. Conduct a dose range finding study of **SW-100** and the best back-up compound from Aim 1 to identify a dose in mouse chow that causes maximal CNS impact and is well tolerated.
Aim 3. Test **SW-100** and the back-up analog from Aim 1 in Tg4510 mice starting at two ages to ascertain the extent to which these new chemical entities can retard the development of the tau phenotype, and whether benefits can be observed even after tau deposition has started. Assessments will thus be made of drug effects on cognition, histological tau deposition, and neurochemical tau accumulation. Any positive effects observed using these drugs after tau deposition would suggest benefit for people who already have dementia.

There are several potential mechanisms by which HDAC6 may produce benefits. First, it may lead to more stable microtubules and enhance axonal transport through increased tubulin acetylation. Second, it may increase tau degradation in the proteasome through increased HSP90 acetylation. Third, it may inhibit tau aggregation through increased tau acetylation. We will monitor acetylation of each of these HDAC6 substrates to begin understanding the mechanism(s) most responsible for benefiting the tau phenotype in this model.

Project Narrative

There are no effective treatments for the accumulation of abnormal proteins, loss of memory and brain shrinkage that occur in Alzheimer's disease and other tauopathy-related neurodegenerative diseases. This project will further establish the ability of brain penetrant HDAC6 inhibitors to serve as potential AD therapies using a relevant mouse model. Success in this project will provide further validation of this therapeutic approach and lay a strong foundation for moving such NCEs to the clinic.

Facilities and Other Resources

StarWise Therapeutics LLC was founded in 2016 and is located in Chicago Illinois.

Laboratory:
We are currently planning to lease 1500 sq. ft. of laboratory space at the Incubator in Skokie Illinois, one of the building previously occupied by Searle Laboratories prior to their acquisition by Pfizer. The laboratory includes all the standard equipment necessary for the daily operation of a medicinal chemistry research laboratory, including sinks, vacuum lines, a rotary evaporators, an analytical balances, a constant temperature bath, and glassware for handling air- and moisture-sensitive materials, fume hoods, HPLC, deionized water, gas and air for full medicinal chemistry operations. Additionally, specialized research facilities including NMR and LC-MS are also available on a fee-for-service basis at Northwestern University.

Office: SWT will have 200 sq. ft. of office space contiguous to the laboratories. Standard office computers, phone lines, etc. are available. All documents and data are backed up daily, and secure. Access to the internet is available through a high-speed T1 line, and journal access will be available through Northwestern University.

Scientific and Business Environment: SWT will have the resources needed to complete the objectives of this investigation by positioning itself in a collaborative environment in terms of expanding the depth and breadth of the company's access to both facilities and thought leaders. There are hundreds of business graduates and students from Northwestern's Kellog School of Business who are anxious to become affiliated with start-up biotech companies and who will be able to offer accounting and business expertise. SWT plans to capitalize on these eager graduates in growing the company.

University of South Florida Resources available to Drs. Gordon and Morgan:

Environment:
The USF Health Byrd Alzheimer's Institute is a 100,000 sf translational research center conducting basic, translational and clinical research related to Alzheimer's disease and related neurodegeneration. Over 30 faculty are housed at the Institute and another 15 are Associate members, producing a rich, interactive scientific community. The Institute houses the university-wide core facilities in electrophysiology, mouse behavior, scanning digital microscopy and viral vector production. Other campus cores include proteomics, flow cytometry, microscopy (confocal, multiphoton, electron and scanning electron) and in vivo animal imaging.

Laboratory:
Laboratory space is located in the USF Byrd Alzheimer Institute. Dr. Gordon operates in a shared, open floor plan lab located on the south side of the 4th floor. The Laboratory consists of over 4,000 sf of laboratory space in 6 double-sided bays as well as 4 biological safety cabinets, a complete mouse stereotaxic surgical suite, two digital scanning microscopes and 4 associated data analysis workstations, and all equipment required for mouse brain histology, nucleic acid chemistry, real-time PCR, western blotting, ELISA, luminex proteomics, cell culture and flow cytometry. Various analysis software packages are available for these studies including Statview, GraphPad, NeuroQuant and Prism. Access is also available to common equipment rooms, cold room and a dark room with X-ray developer located in the Byrd Institute. All equipment needed for this project is conveniently located in close proximity.

Clinical: Although not required for this project, there are 30,000 sf of clinical services and clinical research space within the Institute including a PET Scanner.

Animal:
All animal manipulations for this project will be performed at USF with approval by the USF IACUC. A full service AAALAC-approved vivarium facility is located on the basement floor of the USF Byrd Alzheimer Institute building. Veterinary support is provided by USF. The transgenic mice are housed in rooms dedicated solely for these mice. Two procedure rooms are available containing 2 biological safety cabinets, a fume hood, and an anesthesia vaporizer. The USF Behavior Core facility is housed in an additional 3 rooms dedicated to mouse behavioral testing. Behavioral equipment includes open field apparatus, water mazes, Y maze, Barnes maze, elevated plus maze, rotarod, passive avoidance, and automated fear conditioning apparatus, all with video monitoring, subdued lighting and computer-assisted data analysis. The Behavior Core will be available for use during this project. The Behavior Core serves to consolidate expertise and equipment, and does not charge a fee for service for investigators within the USF Health Byrd Institute.

Computer:
There are many personal computers, printers and other peripheral support for general use, along with full internet and email access. University site licenses are available for major software support. Dedicated PCs also drive numerous major equipment systems, including image analysis workstations.

Office:
The PI and other key personnel each have an office space of 120 sq ft or greater.

StarWise Therapeutics will have all standard chemistry laboratory equipment that includes:

Balances
Buchi Rotary evaporators
Vacuum pumps
Parr Pressure equipment
Shimadzu LC-MS analytical system;
Shimadzu HPLC Prominence analytical systems with PDA and ELSD (light scattering) detectors
Shimadzu Prep HPLC system with UV detector
Agillent 1100 HPLC with UV detector
ISCO CombiFlash Companion 4x purification system
ISCO CombiFlash RF purification system
Shimadzu FT-IR spectrometer; Autopol IV Polarimeter
LabTech Multiple Organic Synthesizer for parallel syntheses
Biotage Discovery Microwave system
Sanyo U730 -30 °C Biomedical Freezer
Thermo Revco -20 °C Freezer
Labconco Cascade Freeze Dry System -84 °C

Equipment available to Dr. David Morgan at University of South Florida:

The Byrd Institute Alzheimer's Research Laboratories contain 4 biological safety cabinets and 4 incubators; a complete mouse surgical suite with gas anesthesia; a Mirax UV-VIS Digital Scanning Microscope, a Zeiss Z1 Digital Scanning microscope and associated data analysis workstations; 2 American Oprtical 860 sliding microtomes and piezo-electruc freezing stages; High throughput immunostaining equipment (240 sections incubated in parallel); BioRad T100 and C1000 thermal cyclers, MJ Research Opticon Real time PCR instrument; Olympus BX51 UV Vis microscope with Evolution MD Digital Camera, Nikon Microphot FX UV Vis microscope with Sony DXC 960 video camera.

Detection devices include a BioRad Magpix Multiplex ELISA reader;Accuri C6 Flow cytometer; Cytation -3 UV VIS luminescence plate reader; Amersham Imager 600 Chemiluminescence Detector; LiCor Odyssey near infrared detector; Protein Simple UV-VIS gel Documentation device; Biotek Plate Washer. Two ultracentrifuges, two J30 Avanti centrifuges and two J6 centrifuges are available within the Institute. Various analysis software packages are available for these studies including Statview, GraphPad, NeuroQuant and Prism. Access is also available to the common equipment rooms, cold room and a dark room with X-ray developer located in the Byrd Institute.

Equipment available to Dr. Marcia Gordon at University of South Florida:

The lab houses one Zeiss Axio-scan and one Mirax digital scanning microscope for collection of permanent, high resolution (200-400x) images of 1x3 inch slides, which is supported by 4 computer work stations with 32in monitors for image processing and TB hard drive storage. Two Stereologer systems consisting of brightfield microscopes with high numerical aperture lenses, motorized stages and computer workstations are located on the 5th floor of the USF Byrd Alzheimer Institute, and will be available for this project. Staff members are fully trained in their operation. Other major equipment housed within the 4th floor South Laboratory that will be utilized for this project includes 2 Bio-Rad thermal cyclers, MJ Research real time thermal cycler, nanopdrop spectrophotometer, 2 sliding microtomes with Peltier-cooled stages and chilled water circulators, power supplies, gel electrophoresis and documentation systems, LiCor Odyssey, plate reader, and J-30 and J-6 refrigerated centrifuges.

The U.S. Small Business Administration

SBIR.gov SBC Registration

SBC Control ID:	[redacted]		
Company Name:	Starwise Therapeutics LLC		
Address:	[redacted]		
City:	CHICAGO		
State:	IL	**Zip:**	60614
EIN (TIN):	[redacted]	**DUNS:**	[redacted]
Company URL:	Starwise therapeutics		
Number of Employees:			[redacted]
Is this SBC majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?			No
What percentage (%) of the SBC is majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?			0.00%

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Alan Middle Name Paul Last Name*: Kozikowski Suffix:

Position/Title*: CEO
Organization Name*: StarWise Therapeutics LLC
Department:
Division:
Street1*: [redacted]
Street2:
City*: Chicago
County: Cook
State*: IL: Illinois
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: [redacted]

Attach Biographical Sketch*: File Name: 1236-APK biosketch.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT12375369 Funding Opportunity Number: PAS-17-065 . Received Date: 2017-03-31T12:25:30.000-04:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Marcia Middle Name Last Name*: Gordon Suffix:

Position/Title*: Professor
Organization Name*: University of South Florida
Department: Molecular Pharmacology and Phy
Division: Morsani College of Medicine
Street1*: [redacted]
Street2:
City*: Tampa
County: Hillsborough
State*: FL: Florida
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted]

[redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: [redacted]

Attach Biographical Sketch*: File Name: 1237-Biosketch_Gordon, Marcia.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: David Middle Name Last Name*: Morgan Suffix:

Position/Title*: Professor
Organization Name*: University of South Florida
Department: Molecular Pharm and Physiology
Division: College of Medicine
Street1*: [redacted]
Street2:
City*: Tampa
County: Hillsborough
State*: FL: Florida
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: DMORGAN!

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: [redacted]

Attach Biographical Sketch*: File Name: 1238-Biosketch_Morgan, David.pdf

Attach Current & Pending Support: File Name:

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Kozikowski, Alan P.

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Professor and CEO

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Michigan, Ann Arbor, Michigan	B.S.	[redacted]	Chemistry
Univ. of California, Berkeley	Ph.D.	[redacted]	Organic Chemistry
Harvard University, Cambridge, MA	Post-Doctoral	[redacted]	Synthetic Org. Chemistry

A. Personal Statement

Dr. Kozikowski's group was the first to synthesize Huperzine A, an alkaloid which he pushed to phase II clinical trials for Alzheimer's disease, and which is now under study as an antidote against nerve gas toxins. He has also developed a potential medication for use in cocaine abuse (Nocaine) and other novel transporter ligands [triple reuptake inhibitors] for use in the therapy of mood disorders. Moreover, based upon a natural product lead, his group has generated novel GSK-3β inhibitors for use in bipolar disorder and for brain cancer. The start-up company Actuate Therapeutics Inc. was recently formed in order to raise venture funding to advance the lead candidate to human clinical trials for use in GBM cancers. The compound will enter clinical trials this year. Furthermore, he has discovered unique inhibitors of the NAAG peptidase (PSMA), which are in Phase II and III clinical trials for prostate cancer imaging. He has also discovered improved versions of these PSMA inhibitors which are being studied for use in photodynamic therapy. His group has designed drug leads for use in tuberculosis and Mycobacteria abscessus, the latter of which is associated with cystic fibrosis. The TB Global Alliance is helping to advance one of these NCEs and has initiated scaleup work and advanced dog cardiotoxicity testing. Furthermore, efforts are now being made to launch a clinical trial for the TB drug in India. Lastly, his interest in epigenetics has led to the discovery of highly selective (>1000-fold), nM potency HDAC6 inhibitors that are being studied in colitis, transplant medicine, Rett syndrome, CMT, cancer, and Alzheimer's disease, which is the subject of this grant application. His experience in the science of drug discovery and development is extensive, and thus he is eminently qualified to lead the present grant program.

B. Positions and Honors

1976-1990	University of Pittsburgh, Professor of Chemistry with adjunct appointment in Behavioral Neuroscience
1990-1993	Mayo Clinic, Professor and Director of Chemistry Research
1995-2003	Professor and Director, Drug Discovery Program, Dept. of Neurology, Georgetown University
2002-present	CEO and Founder, Acenta Discovery, Inc., Tucson, Arizona
2003-present	Adjunct Professor, Johns Hopkins University School of Medicine, Dept. of Pharmacology and Molecular Sciences
2003-present	Professor, Drug Discovery Program, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago

2105-present Founder, Actuate Therapeutics Inc., the brain cancer company
2016-present Founder and President, Vela Therapeutics, LLC, HDAC Company
2016-present Founder and CEO, StarWise Therapeutics LLC

Honors: Phi Beta Kappa; NSF Predoctoral Fellow (1971-1974); NIH Postdoctoral Fellow (1974-1976); Sloan Foundation Fellow (1978-1980); Camille and Henry Dreyfus Teaching Scholar (1982-1987); Ciba-Geigy Award (1982); Japan Society for the Promotion of Science Fellow (1984); Akron ACS Awardee, 1989; Johnson & Johnson Focused Giving Grant Awardee (1988). MDCN5 Study Section, 1998-2000. BDCN NIH SBIR study section, 2002-present. Editorial Advisory Board for Current Topics in Medicnal Chemistry, Current Pharmaceutical Design, Current Medicinal Chemistry; International advisory board of ChemMedChem, published by Wiley-VCH starting 2006, appointed, editorial advisory board, J. Medicinal Chemistry, SAB for the Int. Rett Syndrome Foundation; Founding Advisory Board member of K-RITH, funded by Howard Hughes Medical Institute, Durban, S. Africa. Voldeng Memorial Lecture, MALTO Meeting, Univ. of Mississippi, 2015.

C. Contributions to Science

While Dr. Kozikowski continues to identify small molecules working at novel targets that may be of value in treating Alzheimer's disease, his early efforts in this arena resulted in the advancement of the natural product huperzine A to the clinic. The Phase II clinical studies were sponsored by the NIA and a private company based in NY which Dr. Kozikowski helped to found. He has created a process for preparing huperzine A which has been used to prepare GMP material, and devised analogs of greater potency that have been investigated for use as protective agents from nerve gas toxins. These later results were particularly exciting, as a combination of huperzine A with a benzodiazepine is able to completely block death in rodents exposed to organophosphates, work that is of substantial interest to the Department of Defense. It is of interest to note that because of this work, huperzine A can be found on the shelves of dietary supplements stores like GNC.

1.Xia, Y. and Kozikowski, A. P. (1989). "A Practical Synthesis of the Chinese Nootropic Agent Huperzine-a - a Possible Lead in the Treatment of Alzheimers-Disease." J. Amer. Chem. Soc. 111(11): 4116-4117. PMID not available.

Dr. Kozikowski has pioneered new chemical tools, therapeutics, and imaging agents in a wide variety of scientific areas. For example, working with various collaborators in the cancer and neuroscience fields, Dr. Kozikowski and his team invented a new class of inhibitors of the enzyme GCPII, also known as NAAG peptidase. Of particular value is the possible application of his compounds to prostate cancer, a disease for which there exists no effective treatments. NAAG peptidase is highly homologous to prostate specific membrane antigen or PSMA which is over-expressed in prostate tumors. His urea based inhibitors of PSMA appear to be ideal for imaging prostate tumors by PET methods, work which has now been published in collaboration with the Hopkins PET imaging group. His own research group did all of the original design and synthesis work to create these nanomolar inhibitors, and produced cold/unlabeled versions of the compounds to ensure their utility for PET. A published Phase I human trial showed that these urea-based imaging tools are completely predictive of the presence of prostate cancer in man. Moreover, the National Cancer Institute is currently recruiting patients to examine this agent further, to further advance the utility of the radiotracer **18F-DCFBC** to identify sites of prostate cancer in the human body. An examination of the literature will reveal that the Kozikowski urea-based inhibitors have been used as the starting point in a number of imaging and therapeutic endeavors by a host of other investigators in the US and abroad. These agents were patented when he was at Georgetown University Medical Center, and new patent applications have now been filed by Dr. Kozikowski, as improved agents having sub-nM potency at PSMA have been identified.

2. Banerjee, S. R.; Foss, C. A.; Castanares, M.; Mease, R. C.; Byun, F.; Fox, J. J.; Hilton, J.; Lupold, S. E.; Kozikowski, A. P.; Pomper, M. G. (2008) Synthesis and evaluation of technetium-99m- and rhenium-labeled inhibitors of the prostate-specific membrane antigen (PSMA). J. Med. Chem. 51(15), 4504-17. PMC3336105
3. Zhou, J., Neale, J. H. et al. (2005) NAAG Peptidase Inhibitors and their Potential for Diagnosis and Therapy. Nature Reviews Drug Discovery, 4 (12), 1015-26. PMID: 16341066
4. Cho, S.Y., Gage, K.L., Mease, R.C., Senthamizhchelvan, S., Holt, D.P., Jeffrey-Kwanisai, A., Endres, C.J., Dannals, R.F., Sgouros, G., Lodge, M., Eisenberger, M.A., Rodriguez, R., Carducci, M.A., Rojas, C., Slusher, B.S., Kozikowski, A.P., Pomper, M.G. (2012) Biodistribution, tumor detection, and radiation dosimetry of 18F-DCFBC, a low-molecular-weight inhibitor of prostate-specific membrane antigen, in patients with metastatic prostate cancer. J Nucl Med. 53,

12, 1883-91. PMC3742115

Dr. Kozikowski is working in the hot field of epigenetics, and specifically on the design of inhibitors of the histone deacetylases (HDACs). His group invented one of the most selective HDAC6 agents to have been reported to date. This HDAC6 inhibitor is known as Tubastatin A [a research tool that has been used by a large cadre of investigators, with over 8000 Google entries, with numerous articles using the compound in explorations of HDAC6 in human biology.] In collaboration with the van den Bosch team in Belgium, this agent was found to reverse the phenotype of transgenic mice that contain a mutation in HSPB1, a mutation that leads to symptoms of Charcot Marie Tooth Disease (the largest of peripheral neuropathies; see wild type and tg animals at left). A number of other HDAC6 reagents created by the Kozikowski group are being explored for a host of other applications, including Alzheimer's disease, transplant medicine [they prolong the lifespan of mice with transplanted hearts!], cancer, Rett syndrome, and stroke. A recent paper with Lucas Pozzo-Miller underscores the ability of these HDAC6 inhibitors to facilitate BDNF trafficking. Several of the key HDAC6 patents created at UIC have been licensed by the Acetylon Company, an HDAC company located in Boston; recently certain assets of this company have been acquired by Celgene and thus these patents now belong to Celgene. It should be noted, however, in the context of this grant that the key NCEs to be studied have been patented by Dr. Kozikowski at the uic, and that this patent has been licensed by StarWise Therapeutics.

5. Butler, K. V.; Kalin, J.; Brochier, C.; Vistoli, G.; Langley, B.; Kozikowski, A. P. (2010) Rational Design and Simple Chemistry Yield a Superior, Neuroprotective HDAC6 Inhibitor, Tubastatin A. J. Am. Chem. Soc. 132, 10842-46. PMC2916045
6. d'Ydewalle, C.; Krishnan, J.; Chiheb, D. M.; Van Damme, P.; Irobi, J.; Kozikowski, A. P.; Vanden Berghe, P.; Timmerman, V.; Robberecht, W.; Van Den Bosch, L. (2011) HDAC6 inhibitors reverse axonal loss in a mouse model of mutant HSPB1-induced Charcot-Marie-Tooth disease. Nature Medicine, 17, 968-974. PMID: 21785432
7. Xu, X.; Kozikowski, A.P.; Pozzo-Miller, L. (2014) A selective histone deacetylase-6 inhibitor improves BDNF trafficking in hippocampal neurons from Mecp2 knockout mice: implications for Rett syndrome. Front Cell Neurosci. 8:68. PMC3945638

Moreover, Dr. Kozikowski has pioneered some exciting research in the area of GSK-3 inhibitor design, by inventing novel compounds based on the natural product staurosporine, a molecule that functions as a potent, but non-selective inhibitor of multiple kinases. His efforts have shown that some of his synthetic inhibitors have much higher kinase selectivity, and are able to work like lithium in animal models of mania, thus providing further support for GSK-3 as the target of lithium's action in the treatment of mood disorders. Additionally, his work on the design of safe GSK-3 inhibitors has led to compounds that readily penetrate the BBB, and are able to block mania in *Clock* mutant animals, which have disrupted circadian rhythms.

8. Gunosewoyo H, Midzak A, Gaisina IN, Sabath EV, Fedolak A, Hanania T, Brunner D, Papadopoulos V, Kozikowski AP. Characterization of maleimide-based glycogen synthase kinase-3 (GSK-3) inhibitors as stimulators of steroidogenesis. J Med Chem. 2013 Jun 27;56(12):5115-29. doi: 10.1021/jm400511s. PMC3777810
9. Kozikowski, A. P. et al. (2011) Identification of a maleimide-based glycogen synthase kinase-3 (GSK-3) inhibitor, BIP-135, that prolongs the median survival time of Δ7 SMA KO mouse model of spinal muscular atrophy. ACS Chem. Neuroscience 3, 5-11. PMC3279955
10. Kozikowski, A. P., Gaisina, I. N. et al. (2007) Structure-Based Design Leads to the Identification of Lithium-Mimetics that Block Mania-Like Effects in Rodents – Possible New GSK-3β Therapies for Bipolar Disorders. J. Am. Chem. Soc. 129(26), 8328-32. PMID: 17552518

However, of even greater interest in the GSK-3 arena are his collaborative efforts with colleagues at Northwestern in which the chemistry-biology team has shown that these inhibitors, patented by Dr, Kozikowski with UIC OTM, are able to cause complete regression of GBM brain tumors and pancreatic tumors in PDX mouse models, likely through the regulation of NFκB binding to gene promoters. One of these extensively tested inhibitors is able to significantly prolong the lifespan of animals bearing GBM tumors when used in

combination with the known anti-cancer drug irinotecan. Because of the significance of these findings, combined with convincing ADMET data, an investment group out of the Dallas area has committed financing of a start-up brain cancer company, Actuate Therapeutics Inc. This team from UIC, NU, and Texas has recently met with clinicians who are ready to assist in taking the drug to the clinic. Through the for-profit structure of Actuate Therapeutics, plans have been made to mount the first Phase I clinical studies on the key UIC patented compound in 2017.

Gaisina, I. N.; Gallier, F.; Ougolkov, A. V.; Kim, K. H.; Kurome, T.; Guo, S. P.; Holzle, D.; Luchini, D. N.; Blond, S. Y.; Billadeau, D. D.; Kozikowski, A. P. (2009) From a Natural Product Lead to the Identification of Potent and Selective Benzofuran-3-yl-(indol-3-yl)maleimides as GSK-3*beta* Inhibitors that Suppress Proliferation and Survival of Pancreatic Cancer Cells. J. Med. Chem. 52(7),1853-63. PMC2665923

In the field of drug abuse, Dr. Kozikowski has published on a host of novel ligands working at dopamine, norepinephrine, and serotonin transporters. His group has identified some of the most potent and selective ligands for both the SERT and NET transporters, which have been investigated by researchers in a number of institutes including the NIMH for use in brain imaging by PET. Of substantial importance to the drug abuse field are his contributions to the identification of a possible medication for use in cocaine addiction. He has found DAT/NET selective ligand, Nocaine, that is less reinforcing than cocaine in primate studies, and which is capable of blocking some of cocaine's locomotor stimulant and convulsant effects. Along these same lines, Dr. Kozikowski has more recently invented a new class of highly selective $5HT_{2c}$ ligands that are being advanced for the treatment of schizophrenia. These compounds have proven to block the action of amphetamine in open field tests, to be effective in pre-pulse inhibition studies, and to show positive effects in the novel object recognition assay using NR1-knockdown mice. These compounds are being further studied to treat substance abuse, and in particular, methamphetamine addiction.

11. Cheng J; Giguère PM; Onajole OK; Lv W; Gaisin A; Gunosewoyo H; Schmerberg CM; Pogorelov VM; Rodriguiz RM; Vistoli G; Wetsel WC; Roth BL; Kozikowski AP (2015) Optimization of 2 phenylcyclopropyl-methylamines as selective serotonin 2C receptor agonists and their evaluation as potential antipsychotic agents. J Med Chem. 58(4),1992-2002. PMID: 25633969

URL for full list of publications, see: https://www.ncbi.nlm.nih.gov/myncbi/alan.kozikowski.1/cv/69090/

D. Research Support.

ACTIVE

[redacted]

HDACi that do not enter the CNS, as CMT is a peripheral disease. This grant does not overlap with the present proposal focusing on the design of new agents for cancer, as the chemical scaffolds being pursued are different, as are the majority of the biological endpoints.

COMPLETED

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Marcia N. Gordon, PhD

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Professor of Molecular Pharmacology and Physiology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Southern California, Los Angeles, CA	B.S.	[redacted]	Psychobiology
University of Southern California, Los Angeles, CA	PhD	[redacted]	Molecular Biology
University of California, Los Angeles, CA	Postdoc	[redacted]	Neuroscience

A. Personal Statement. I have a long-standing interest in the role of aging and neuroinflammation on brain function. I have been engaged in the study of brain aging and Alzheimer's research for more than 15 years. I am especially interested in the role of innate immunity in aging and neurodegenerative disease. Increasingly, my research focuses on developing novel therapeutics to modulate innate immunity, including small molecule agents, vaccines and biologics. I served as PI on an R01 level award for 14 years (AG15490) and am a recent recipient of the Zenith Award from the Alzheimer's Association. I have also managed corporate contracts, served as co-investigator on federal awards, and have published over 100 articles, with an H index of 36. Specific to this project, I have extensive experience with maintaining colonies of aged rodent and transgenic mouse models of Alzheimer's disease, and with administration of therapeutic agents to rodents, including via alternative delivery routes. The following publications document my long-standing experience with alternative modes of drug delivery. I have considerable experience with phenotyping mutant rats and mice at behavioral and histological levels, especially as it pertains to brain development, cognitive function and aging. My laboratory routinely performs a wide range of behavioral testing in mice. I was instrumental in adapting the radial arm water maze for use in mice, and was a co-author of the first report that anti-amyloid immunotherapy improved cognitive performance in transgenic mice with amyloid deposition. Immunostaining, in situ hybridization, stereology and computer-assisted image analysis are widely employed by my lab, and I have published several papers and book chapters describing relevant methodology. Consequently, I have the necessary expertise and leadership to serve as principal investigator for the subcontractor of this project.

- Selenica ML, Benner L, Housley SB, Manchec B, Lee DC, Nash KR, Kalin J, Bergman JA, Kozikowski A, **Gordon MN**, Morgan D (2014) Histone deacetylase 6 inhibition improves memory and reduces total tau levels in a mouse model of tau deposition. Alzheimers ResTher 6:12, PMID: 24576665, PMCID: PMC3978441.
- Wilcock DM, Jantzen PT, Li Q, Morgan D, **Gordon MN** (2007) Amyloid-β vaccination, but not nitro-nonsteroidal anti-inflammatory drug treatment, increases vascular amyloid and microhemorrhage while both reduce parenchymal amyloid. Neuroscience 144: 950-960, PMID: 17137722, PMCID: PMC1857306.
- **Gordon MN**, Muller CD, Sherman KA, Morgan DG, Azarro AJ, Wecker L (1999) Oral versus transdermal selegiline: Antidepressant activity in the rat. Pharmacology, Biochemistry and Behavior 63: 501-506, PMID: 10418793.

B. Positions and Honors.

Positions and Employment:

1989-1992 **Research Assistant Professor**; Univ. of Southern California, Andrus Gerontology Center.
1992-2000 **Assistant Professor**; Univ. of South Florida, Dept. of Pharmacology and Therapeutics.
2000-2005 **Associate Professor**; Univ. of South Florida, Dept. of Pharmacology and Therapeutics.
2005- present **Professor**; Univ. of South Florida, Dept. of Molecular Pharmacology and Physiology.

Honors and Awards:

1978-1982 **Teaching Assistant Fellowship**; Dept. of Biological Sciences, Univ. of Southern Cal.
1982-1985 **Predoctoral Fellowship**; Andrus Gerontology Center, Univ. of Southern California; NIA Training Grant in Endocrinology and Neurobiology of Aging; Mentor: Caleb E. Finch
1985-1987 **Postdoctoral Fellowship**; Mental Retardation Research Center, UCLA; NICHHD Training Grant in Mental Retardation; Mentor: Jean de Vellis, PhD.
1987 **Biomedical Research Support Grant Award**; Neuropsychiatric Institute, UCLA.
1987-1989 **Postdoctoral Fellowship**; Dept. of Anatomy and Brain Research Institute, UCLA; NICHHD Training Grant in Neuroendocrinology; Mentor: Jean de Vellis, PhD.
1989 **Travel Award for Young Investigators**; American Society for Neurochemistry.
1992 **Travel Award**; University of Michigan Claude D. Pepper Geriatrics Research and Training Center Scholarship.
1992 **American Federation for Aging Research Award for Biomedical Research**
1997 **Research and Creative Scholarship Award**; Univ. of South Florida Research Council
1998-2010 Ad hoc **Peer Review Panelist**; BBBP-E; F01; BDCN; NSD-C; BINP.
2010-2012 **STEP-UP Mentor;** National Institute of Diabetes and Digestive and Kidney Diseases Short-Term Education Program for Underrepresented Persons; Jasmine Roberts (high school program, 2010-2011; undergraduate program, 2012).
2014 **Zenith Award**, Alzheimer's Association

C. Contribution to Science

A complete listing of publications may be viewed at MyBibliography: http://www.ncbi.nlm.nih.gov/sites/myncbi/marcia.gordon.1/bibliography/40628629/public/?sort=date&direction=ascending

1. The cluster of my research publications that has been the most cited involves phenotyping and immunotherapy of amyloid-depositing transgenic mice. The first transgenic mouse that recapitulated amyloid deposition with a process similar to that of human patients with Alzheimer's disease was produced by Karen Hsiao Ashe in the mid1990s. She contributed some of these mice to collaborators at USF, notably John Hardy. Shortly thereafter, Hardy's lab generated transgenic mice carrying mutations in presenilin-1 that were linked to Alzheimer's disease. The Hardy group established a collaboration with Dave Morgan and myself. As a junior faculty member, I was personally "in the trenches," performing experiments at the bench. I personally established the first breeding colonies and crossed the lines to develop mice carrying two genetic mutations linked to Alzheimer's disease. I still maintain this colony, some 20 years later. I performed immunostaining to characterize the neuropathology, and contributed to developing a behavioral battery to assess learning and memory capacity, among other skills. I was also instrumental in the performing the first immunotherapy experiments performed by our group, personally preparing the injectate, injecting and monitoring the mice, and conducting neuropathological assessments. We were the first group to demonstrate cognitive benefits in amyloid-depositing mice after active anti-amyloid vaccination.

   a. Duff K, Eckman C, Zehr C, Yu X, Prada CM, Perez-tur J, Hutton M, Buee L, Harigaya Y, Morgan D, **Gordon MN**, Holcomb L, Refolo L, Zenk B, Hardy J, Younkin S (1996) Increased amyloid-β42(43) in brains of mice expressing mutant presenilin 1. Nature 383: 710-713, PMID: 8878479, number of citations: 1158.
   b. Morgan, D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, Duff K, Jantzen P, Di Carlo G,Wilcock D, Connor K, Hatcher J, Hope C, **Gordon MN,** Arendash GW (2000) Aß peptide vaccination prevents memory loss in and animal model of Alzheimer's disease. Nature 408: 982-985, PMID: 11140686, number of citations: 1181.

c. Holcomb L, **Gordon MN** (joint first authorship), McGowan E, Yu X, Benkovic S, Jantzen P, Wright K, Saad I, Mueller R, Morgan D, Sanders S, Zehr C, O'Campo K, Hardy J, Prada CM, Eckman C, Younkin S, Hsiao K, Duff K (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. Nature Medicine 4: 97-100, 1998, PMID: 9427614, number of citations: 903.

d. **Gordon MN**, Holcomb LA, Jantzen P, Di Carlo G, Wilcock D, Boyett KW, Connor K, Melachrino J, O'Callaghan JP, Morgan D (2002) Time course of the development of Alzheimer-like pathology in the doubly transgenic PS1+APP mouse. Experimental Neurology 173: 183-195 (with cover art), PMID: 11822882, number of citations: 165.

2. It became clear during this work that amyloid-depositing mice do not develop frank neurodegeneration (described in publication 1d above). Although dystrophic neurites are present, we were not able to measure reductions in synaptic markers, to visualize dying TUNEL-positive neurons, or to detect any aspect of brain atrophy. The inflammation hypothesis of Alzheimer's disease was prominent at the time, which posits that activation of the immune axis could induce amyloid precursor protein, leading to a vicious cycle, ultimately causing neurodegeneration. I proposed in the first R01 grant that I received that I could provoke neurodegeneration in the context of amyloid deposition by injecting the canonical immune activator, lipopolysaccharide (LPS). In contrast to this original hypothesis, my lab discovered that the injection of LPS activated microglial cells to phagocytose amyloid, removing it from the parenchyma, and restoring memory function. These results have now been corroborated by numerous investigators using a variety of agents to activate microglia. We have also examined other agents to activate microglia in this model. More recently, we demonstrated that the same treatments that reduce amyloid exacerbate tauopathy in different transgenic mice with over-expression of tau. These results may explain why global suppression of inflammation does not benefit Alzheimer patients.
   a. Herber DL, Maloney JL, Roth LM, Freeman MJ, Morgan D, **Gordon MN** (2006) Diverse microglial responses after intrahippocampal administration of lipopolysaccharide. Glia 53: 382-391, PMID: 16288481.
   b. Herber DL, Mercer M, Roth LM, Symmonds K, Maloney J, Wilson N, Freeman MJ, Morgan D, **Gordon MN** (2007) Microglial activation is required for Aβ clearance after intracranial injection of lipopolysaccharide in APP transgenic mice. Journal of Neuroimmune Pharmacology 2: 222-231, PMID: 18040847.
   c. Lee DC, Rizer J, Hunt JB, Selenica ML, **Gordon MN**, Morgan D (2013) Experimental manipulations of microglia in mouse models of Alzheimer's pathology. Activation reduces amyloid but hastens tau pathology. Neuropathology and Applied Neurobiology 39:69-85, PMID: 23171029, PMCID: PMC4300851.
   d. Selenica MLB, Alvarez JA, Nash KR, Lee DC, Cao C, Lin X, Reid P, Mouton PR, Morgan D, **Gordon MN** (2013) Diverse activation of microglia by chemokine (C-C motif) ligand 2 overexpression in brain. Journal of Neuroinflammation 10: 86, PMID: 23866683, PMCID: PMC3726363.

3. Another major area of interest is in the role of aging in neuroinflammation and glial function. Alzheimer's disease occurs in the context of the aging brain, and no symptoms develop when patients are young. Many years ago, I discovered that glial cells in the aging brain respond to damage, surprisingly, with exaggerated reactivity. We later showed that the exaggerated reactivity only occurs in response to proinflammatory (M1) stimuli. The aging brain loses the ability to respond to anti-inflammatory (M2) stimuli. The fundamental goals of my current Zenith Award grant are to boost anti-inflammatory function in the aging brain using gene therapy.
   a. **Gordon MN**, Schreier WA, Holcomb LA, Ou X, Morgan DG (1997) Exaggerated astrocyte reactivity after nigrostriatal deafferentation in the aged rat. Journal of Comparative Neurology 388: 106-119, PMID: 9364241.
   b. Li Q, Lebson L, Lee DC, Nash K, Grimm J, Rosenthal A, Selenica M-LB, Morgan D, **Gordon MN** (2012) Chronological age impacts immunotherapy and monocyte uptake independent of amyloid load. Journal of Neuroimmune Pharmacology 7: 202–214, PMID: 22198698.
   c. Lee DC, Ruiz CR, Lebson L, Selenica ML, Rizer J, Hunt JB Jr, Rojiani R, Reid P, Kammath S, Nash K, Dickey CA, **Gordon MN**, Morgan D. (2013) Aging enhances classical activation but mitigates

alternative activation in the central nervous system. Neurobiology of Aging 34: 1610-1620, PMID: 23481567, PMCID: PMC3652232.

4. The ultimate goal of my research program is translation of an appropriate therapy to human patients. My lab has been testing specific anti-inflammatory therapies in transgenic mouse models with selected components of Alzheimer-like pathology. We have tested small molecules in the past. We have also explored caloric restriction and various diet components. More recently, we are using immunotherapy to modulate innate immunity. This was the basis of a corporate contract which has just concluded, with manuscripts in preparation.
   a. Jantzen, PT, Connor KE,Di Carlo G, Wenk GL, Wallace JL, Rojiani AM, Coppola D, Morgan D, **Gordon MN (**2002) Microglial activation and ß-amyloid deposit reduction caused by a nitric oxide-releasing nonsteroidal anti-inflammatory drug in amyloid precursor protein plus presenilin-1 transgenic mice. Journal of Neuroscience 22: 2246-2254, PMID: 11896164.
   b. Ambegaonkar MU, Nagle AS, Breitner JC, Deleon J, Alamed J, Wilson N, Morgan D, **Gordon MN** (2004) The histamine H2-receptor antagonist cimetidine activates microglia in APP+PS1 transgenic mice. Brain Aging 4: 35-40.
   c. Wilcock DM, Jantzen PT, Li Q, Morgan D, **Gordon MN** (2007) Amyloid-β vaccination, but not nitro-nonsteroidal anti-inflammatory drug treatment, increases vascular amyloid and microhemorrhage while both reduce parenchymal amyloid. Neuroscience 144: 950-960, PMID: 17137722, PMCID: PMC1857306.

5. Finally, a major direction my lab is moving is toward gene therapy. Together with Kevin Nash, I have begun using gene therapy to modulate innate immunity as a therapeutic strategy in mouse models with Alzhiemer-like pathology.
   a. Lebson L, Nash K, Kamath S, Herber D, Carty N, Lee D, Li Q, Szekeres K, Jinwal U, Koren J, Dickey CA, Gottschall P, Morgan D, **Gordon MN** (2010) Trafficking CD11b-positive blood cells deliver therapeutic genes to the brain of amyloid depositing transgenic mice, Journal of Neuroscience 30: 9651–9658, PMID: 20660248, PMCID: PMC2929651. This paper was featured in the column “This Week in the Journal” (http://www.jneurosci.org/content/30/29/i.full).
   b. Carty N, Nash KR, Brownlow M, Cruite D, Wilcock D, Selenica ML, Lee DC, **Gordon MN**, Morgan D. Intracranial injection of AAV expressing NEP but not IDE reduces amyloid pathology in APP+PS1 transgenic mice (2013). PLoS One e59626, PMID: 23555730, PMCID: PMC3610740.
   c. Selenica MLB, Alvarez JA, Nash KR, Lee DC, Cao C, Lin X, Reid P, Mouton PR, Morgan D, **Gordon MN** (2013) Diverse activation of microglia by chemokine (C-C motif) ligand 2 overexpression in brain. Journal of Neuroinflammation 10: 86, PMID: 23866683, PMCID: PMC3726363.

D. Research Support

Ongoing Research Support

[redacted]

[redacted]

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: David Morgan

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Distinguished Professor of Molecular Pharmacology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Northwestern University, Evanston IL	BA	[redacted]	PHILOSOPHY/PSYCHOLOGY
Northwestern University, Evanston IL	MS	[redacted]	NEUROBIOLOGY
Northwestern University, Evanston IL	PHD	[redacted]	NEUROBIOLOGY
University of Southern California, Los Angeles	POSTDOC	[redacted]	NEUROGERONTOLOGY

A. Personal Statement. Dave Morgan has served as PI and Co-I on a wide range of neuroscience research projects focusing on the chemistry of memory and aging. Since 2009 he has led the Byrd Alzheimer's Institute at USF, a translational research center with both laboratory and clinical research activities. He has studied animal models of Alzheimer's and Parkinson's pathology for 30 years and performed translational research studies of the impact of immunotherapy, gene therapy and inflammation on murine models of amyloid or tau deposition. Dr Morgan will assist Dr Peng by providing expertise regarding research questions in Alzheimer's disease and interpretation of data obtained in this project. His laboratory will also perform the histological analysis of the tissue.

B. Positions and Honors

Research Assistant Professor:	University of Southern California, 1985 - 1988
Assistant Professor:	University of Southern California, School of Gerontology; 1988 - 1992
Associate Professor:	University of South Florida, Department of Pharmacology: 1992-1998
Associate Director:	Institute on Aging, University of South Florida: 1996-2003
Professor	University of South Florida, Dept. of Pharmacology, 1998-2006
	Dept. of Molecular Pharmacology and Physiology 2006 - present
Director	Basic Neuroscience Research, USF College of Medicine 2006-2012
Chief Scientific Officer	Byrd Alzheimer Institute, USF 2009-2012
Chief Executive Officer	Byrd Alzheimer Institute, USF 2010- 2017

HONORS
New Investigator Award in the Neurosciences: American Geriatrics Society; 1985
Nathan Shock New Investigator Award: Gerontological Society of America; 1986
The Anna Greenwall Award: The American Federation for Aging Research; 1987
Established Investigator Award: The American Heart Association; 1989
USF President's Award for Faculty Excellence, 2002
Distinguished USF Health Professor, 2010
Medical Hero Award, Temple Terrace Chamber of Commerce, 2012
Testified on Geroscience at US Senate Select Committee on Aging, 2013

C. Contributions to Science ; (over 180 publications; h factor =57)

1. Demonstration of pyruvate dehydrogenase (PDH) activation during learning in rat brain. As a PhD student with Areyh Routtenberg at Northwestern University I was tasked with attempting to identify phosphoproteins which were changed during learning in rat brain. One experiment I performed used EDTA to chelate magnesium. This resulted in a single band being phosphorylated on the autoradiograms from polyacrylamide gels. Given the molecular weight and the low requirement of PDH kinase for magnesium it seemed very likely this band was PDH. We further demonstrated that the activity of PDH was also modified during training using $^{14}CO_2$ capture assays. This work was published in Science and several other manuscripts. Over 10 years later Paul Gold demonstrated that one action of glucose mediated regulation of memory was via PDH activation.

Morgan, D.G. and A. Routtenberg (1981) Brain pyruvate dehydrogenase phosphorylation and enzyme activity altered by a training experience. **Science**, 214: 470-471.

Morgan, D.G. and A. Routtenberg (1982) Brain pyruvate dehydrogenase activity. Regulation by phosphorylation-dephosphorylation. Brain Research, 251: 391-394.

2. A second major observation regarded work on changes in glial responses to brain injury with normal aging. This work was conducted initially with Tuck Finch at University of Southern California. In addition to work with neurotransmitter receptor regulation, we observed a dramatic increase in GFAP RNA with aging in brain, and further increases when animals would enter premorbid agonal states. Moreover, the response to neuronal deafferentation in the aged brain, while delayed relative to young and middle-aged mice, was exaggerated and prolonged. Ultimately we demonstrated similar changes in microglia in response to deafferenting lesions. These observations are consistent with the subsequent notion that aging results in heightened sensitivity of the innate immune system to activating stimuli, a condition termed “inflammaging”.

Goss, J.R., C.E. Finch and **D.G. Morgan** (1991). Age-related changes in glial fibrillary acidic protein RNA in the mouse brain. Neurobiol. Aging 12:165-170. (120)

Goss, J.R., C.E. Finch and **D.G. Morgan** (1990). GFAP RNA increases during a wasting state in old mice. Exp. Neurol. 108:266-268.

Goss, J.R. and **D.G. Morgan**. (1995) Increased reactivity of glial fibrillary acidic protein following brain injury in aged mice. Journal of Neurochemistry 64:1351-1360.

Gordon, M.N., W.A. Schreier, X. Ou, L.A. Holcomb and **D.G. Morgan**. (1997) Exaggerated astrocyte reactivity after nigrostriatal deafferentation in the aged rat. Journal of Comparative Neurology 388:106-119. (73)

3. Another role was in the development of models of amyloid deposition. This included an early investigation of direct intracranial infusion of amyloid in rats. When combined with perlecan, Abeta infusions made deposits with congophilic properties similar to those in AD brain (published in Neuron). Later work involved collaborations with John Hardy and Karen Duff on their PS-1 transgenic mouse. We then crossed the PS-1 mouse with the APP Tg2576 mouse from Karen Ashe. These mice were characterized in our laboratory and the results were published in Nature Medicine with my PhD student Leigh Holcomb as first author.

Snow, A.D., R. Sekiguchi, D. Nochlin, K. Kimata, A. Mizutami, M. Arai, W.A. Schreier and D. G. **Morgan** (1994). An important role of heparan sulfate proteoglycan (perlecan) in a model system for the deposition and persistence of fibrillar Aβ-amyloid in rat brain. Neuron 12:219-234. (263)

Duff, K. C. Eckman, C-M. Prada, C. Zehr, X. Yu, J. Perez-Tur, M. Hutton, L. Buee, Y. Harigaya, **D. Morgan,** M. Gordon, L. Holcomb, L. Refolo, B. Zenk, J. Hardy, and S. Younkin (1996). Increased amyloid beta 42(43) in brains of mice expressing mutant presenilin-1. **Nature** 383:710-713.

Holcomb, L.A., M.N. Gordon, E. McGowan, S. Benkovic, P. Jantzen, K. Wright, I. Saad, R. Mueller, D. **Morgan***, X. Yu, S. Sanders, C. Zehr, K. O’Campo, J. Hardy, C-M. Prada, C. Eckman, S. Younkin, K. Hsiao, K. Duff (1998). Accelerated Alzheimer-phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. **Nature Medicine** 4:97-101. *corresponding author.

4. A fourth observation was that anti-amyloid immunotherapy could dramatically diminish the amyloid phenotype in amyloid-depositing transgenic mice. We initially had concern that the activation of the innate immune system associated with antigen-antibody complexes might cause a premature memory dysfunction. However, when we performed the vaccination we did not see evidence for early memory loss. However, at later survival times we found protection from the development of memory deficits (this was published in

Nature). We further identified that in aged mice, while antibodies were capable of removing pre-existing amyloid deposits, this resulted in innate immune activation and development of microhemorrhage. These results presaged the amyloid related imaging abnormalities (ARIA) that have plagued human clinical trials of immunotherapy. A critical but under-recognized finding, was our demonstration that diminishing the effector functions of the antibody (via deglycosylation) reduced the development of microhemorrhage, yet still permitted clearance of the amyloid.

Morgan, D., D.M. Diamond, P. Gottschall, K.E. Ugen, C. Dickey, J. Hardy, K. Duff, P. Jantzen, G. DiCarlo, D. Wilcock, K. Connor, J. Hatcher, M. Gordon and G.W. Arendash (2000). Vaccination with Aß Peptide Prevents Memory Deficits in an Animal Model of Alzheimer's Disease. **Nature** 408:982-985.

Wilcock DM, Alamed J, Gottschall PE, Grimm J, Rosenthal A, Pons J, Ronan V, Symmonds K, Gordon MN and, **D Morgan** (2006) Deglycosylated anti-Aß antibodies reverse cognitive deficits, dramatically reduce parenchymal amyloid with minimal vascular consequences in aged APP transgenic mice. **Journal of Neuroscience** 26:5340-46.

5. A fifth emphasis has been on the role of neuroinflammation in regulating both amyloid and tau pathology. Very early we found that LPS acutely administered to brain did not result in neurodegeneration, but instead rapidly cleared amyloid in a mechanisms that could be suppressed by glucocorticoid receptor activation. We and others have now observed that a number of methods of innate immune system activation can clear amyloid. A major concern, however, is that these same manipulations in tau mice lead to acceleration of tau pathology. Our working hypothesis is that innate immune system activations associated with attempts to remove amyloid can initiate or accelerate tau pathology and the progression of Alzheimer's disease.

DiCarlo, G., D. Wilcock, D. Henderson, M. Gordon, and **D. Morgan** (2001). Intrahippocampal LPS injections reduce Aß load in APP+PS1 transgenic mice. Neurobiology of Aging 22: 1007-1012. (117)

Herber DL, Mercer M, Roth LM, Symmonds K, Maloney J, Wilson N, Freeman MJ, **Morgan D**, Gordon MN.(2007) Microglial activation is required for Abeta clearance after intracranial injection of lipopolysaccharide in APP transgenic mice. **J Neuroimmune Pharmacol.**;2:222-31

Lee DC, Rizer J, Hunt JB, Selenica ML, Gordon MN, **Morgan D**. (2012). Experimental manipulations of microglia in mouse models of Alzheimer's pathology. Activation reduces amyloid but hastens tau pathology. **Neuropathol Appl Neurobiol** 39:69-85 PMID: 23171029 PMC4300851

Nash KR, Lee DC, Hunt JB Jr, Morganti JM, Selenica ML, Moran P, Reid P, Brownlow M, Guang-Yu Yang C, Savalia M, Gemma C, Bickford PC, Gordon MN, **Morgan D**. (2013). Fractalkine overexpression suppresses tau pathology in a mouse model of tauopathy. Neurobiol Aging. 2013 34:1540-8.PMID: 23332170

My Bibliography access
http://www.ncbi.nlm.nih.gov/sites/myncbi/david.morgan.2/bibliography/41143698/public/?sort=date&direction=ascending

Recent Publications not listed above

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165) Selenica ML, Benner L, Housley SB, Manchec B, Lee DC, Nash KR, Kalin J, Bergman JA, Kozikowski A, Gordon MN, Morgan D. (2014) Histone deacetylase 6 inhibition improves memory and reduces total tau levels in a mouse model of tau deposition. Alz. Res & Ther. 6:12 (10 pages). PMID: 24576665

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167) Brownlow ML, Joly-Amado A, Azam S, Elza M, Selenica ML, Pappas C, Small B, Engelman R, Gordon MN, Morgan D. (2014). Partial rescue of memory deficits induced by calorie restriction in a mouse model of tau deposition. Behav. Brain Res. 271:79-88. PMID: 24925454

168) Joly-Amado A, Brownlow M, Pierce J, Ravipati A, Showalter E, Li Q, Gordon MN, Morgan D. (2014). Intraventricular human immunoglobulin distributes extensively but fails to modify amyloid in a mouse model of amyloid deposition. Curr. Alzheimer Res. 11:664-671. PMID: 25115543

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175) Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM, Herrup K, Frautschy SA, Finsen B, Brown GC, Verkhratsky A, Yamanaka K, Koistinaho J, Latz E, Halle A, Petzold GC, Town T,Morgan D, Shinohara ML, Perry VH, Holmes C, Bazan NG, Brooks DJ, Hunot S, Joseph B, Deigendesch N, Garaschuk O, Boddeke E, Dinarello CA, Breitner JC, Cole GM, Golenbock DT, Kummer MP. (2015). Neuroinflammation in Alzheimer's disease. Lancet Neurol. 14:388-405.
176) Delic V, Brownlow M, Joly-Amado A, Zivkovic S, Noble K, Phan TA, Ta Y, Zhang Y, Bell SD, Kurien C, Reynes C, Morgan D, Bradshaw PC. (2015). Calorie restriction does not restore brain mitochondrial function in P301L tau mice, but it does decrease mitochondrial F0F1-ATPase activity. Mol Cell Neurosci. 67:46-54.
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185) Joly-Amado A, Serraneau KS, Brownlow M, Marín de Evsikova C, Speakman JR, Gordon MN, Morgan D (2016) Metabolic changes over the course of aging in a mouse model of tau deposition. Neurobiol Aging. 44:62-73

D. Additional Information: Research Support and/or Scholastic Performance

[redacted]

PROJECTS CONCLUDED

[redacted]

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: StarWise Therapeutics LLC

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Alan	Paul	Kozikowski		PD/PI	[redacted]				[redacted]	0.00	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Total Senior/Key Person [redacted]

Additional Senior Key Persons: File Name:

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Secretarial/Clerical						
	Undergraduate Students						
1	Chief Chemist				[redacted]	0.00	[redacted]
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PAS-17-065 . Received Date: 2017-03-31T12:25:30.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: StarWise Therapeutics LLC

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
1. [redacted]	[redacted]
Total funds requested for all equipment listed in the attached file	
Total Equipment	[redacted]

Additional Equipment: File Name:

D. Travel

	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
2. Foreign Travel Costs	[redacted]
Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs

	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	[redacted]
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	[redacted]

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12375369 Funding Opportunity Number: PAS-17-065 . Received Date: 2017-03-31T12:25:30.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: StarWise Therapeutics LLC

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Assay costs for HDAC and ADMET	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . allowable SB rate	40.00	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*
	[redacted]

K. Budget Justification*	File Name: 1234-Starwise_Budget Justification.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12375369 Funding Opportunity Number: PAS-17-065 . Received Date: 2017-03-31T12:25:30.000-04:00

Budget Justification – Chemistry and Biology

Justification of increase in total budget beyond [redacted] Herein we detail our projected expenses for the project. The goal of this project is to expand on our initial exciting findings that HDAC6 inhibitors may have valuable effects as therapeutics in the treatment of Alzheimer's disease. We believe that it is essential to test an improved HDAC6i that has better brain penetrance than Tubastatin A, a compound will low brain access, yet one that showed positive effects in the tau mouse model. In this proposal we will prepare 4 selected analogs of the new lead HDAC6i SW-100 and to advance these through the pre-clinical and eventually clinical stages of development.

StarWise Therapeutics LLC was formed in order to engage in drug discovery efforts targeting neurological disorders including certain peripheral neuropathies, and the company will oversee all aspects of the research to be conducted. All chemistry work will be carried out at the company while the additional HDAC testing will be contracted to Reaction Biology Corporation and the ADMET testing will be outsourced to the CRO Pharmaron.

In order to perform the costly in vivo components of the project we will employ the expertise of Dr. Marcia Gordon who is a leader in the AD field and who is a Professor at the University of South Florida (USF). Dr. Kozikowski has been collaborating for several years with researchers at the Byrd Institute at USF on the application of HDAC6i to Alzheimer's disease. The timely completion of both components, the chemistry and the biology, of the project are essential for its success, thus requiring a slightly higher budget request. The appropriate programmatic and administrative personnel of each organization involved in this grant application are aware of the NIH consortium agreement policy and are prepared to establish the necessary inter-organizational agreement(s) consistent with that policy.

Lastly, as the NIH has received a waiver from SBA, as authorized by the statute, to exceed the hard cap for specific topics as listed in PHS-2016-2.

Personnel – Medicinal Chemistry at StarWise Therapeutics LLC (Biology budget for USF is provided as a separate document)

Alan P Kozikowski, Founder, and CEO of StarWise Therapuetics LLC, [redacted] Dr. Alan Kozikowski is an internationally recognized organic medicinal chemist who has extensive experience in drug discovery for CNS indications. He has published over 520 papers and 100 patents. In order to best accelerate the development of new therapies for AD and CMT, he founded SWT. Dr. Kozikowski will oversee the SWT chemist assigned to the AD project, and he will ensure the constant flow of materials to Dr. Gordon at USF, aid in interpretation of all data, and oversee the acquisition and interpretation of the ADMET data.

Chief Chemist, [redacted] The Chief Chemist will be responsible for the synthesis and characterization of the new HDAC6 inhibitors, and will also be responsible for analyzing tissue samples by LC-MS. Additionally, the Chief Chemist will prepare all of the materials to be shipped to a selected CRO for ADMET assays.

Materials and Supplies – [redacted]

Materials and supplies under these specific categories are requested for this project:

- Chemicals and reagents including NMR solvents and HPLC and NMR machine Time: [redacted]

[redacted]

These costs will cover the assay of 4 new analogs at all 11 HDAC isozymes. We have had considerable success in working with Reaction Biology Corporation in PA and will thus contract these studies to them.

Pharmaron - ADME profiling: [redacted] which includes inter alia the following tests that would be conducted only on the candidates passing required in vitro assays:

- Aqueous solubility
- Enzymatic stability in microsomes
- Caco-2 (A to B and B to A)
- CYP inhibition
- Ames, hERG
- Brain/plasma PK

Indirect costs [redacted]

An overall rate of [redacted] of total direct costs is requested. This amount is appropriate to cover the Avanti current projected indirect costs and is consistent with NIH's policy for Phase I STTR proposals when the company does not already have a previously negotiated indirect cost rate. Rate is also inclusive of indirects charged on the first [redacted] of the consortium agreement to the University of South Florida.

Fee (7%) - [redacted]

A fee of 7% of total costs (direct & indirect) is requested. This fee contributes to the growth of the small business concern by allowing expansion of resources and personnel development. The fee is consistent with a normal profit margin provided for research and development work.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)
Section A, Senior/Key Person	[redacted]
Section B, Other Personnel	[redacted]
Total Number Other Personnel	[redacted]
Total Salary, Wages and Fringe Benefits (A+B)	[redacted]
Section C, Equipment	[redacted]
Section D, Travel	[redacted]
1. Domestic	[redacted]
2. Foreign	[redacted]
Section E, Participant/Trainee Support Costs	[redacted]
1. Tuition/Fees/Health Insurance	[redacted]
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
6. Number of Participants/Trainees	
Section F, Other Direct Costs	[redacted]
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other 1	[redacted]
9. Other 2	
10. Other 3	
Section G, Direct Costs (A thru F)	[redacted]
Section H, Indirect Costs	[redacted]
Section I, Total Direct and Indirect Costs (G + H)	[redacted]
Section J, Fee	[redacted]

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of South Florida

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Marcia		Gordon		PD/PI	[redacted]	2.40			[redacted]	[redacted]	[redacted]
2.	Dr.	David		Morgan		Co-Investigator		0.60					

Total Funds Requested for all Senior Key Persons in the attached file

Total Senior/Key Person [redacted]

Additional Senior Key Persons: File Name:

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits* ($)	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
[redacted]	Technician	[redacted]			[redacted]	[redacted]	[redacted]
[redacted]	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PAS-17-065 . Received Date: 2017-03-31T12:25:30.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: University of South Florida

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel **Funds Requested ($)***

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)
2. Foreign Travel Costs

Total Travel Cost

E. Participant/Trainee Support Costs **Funds Requested ($)***

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees **Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12375369 Funding Opportunity Number: PAS-17-065 . Received Date: 2017-03-31T12:25:30.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: University of South Florida

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	[redacted]
8. Assay costs for HDAC and ADMET	[redacted]
Total Other Direct Costs	

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	49.50	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency [redacted]
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: 1234-Starwise_Budget Justification.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12375369 Funding Opportunity Number: PAS-17-065 . Received Date: 2017-03-31T12:25:30.000-04:00

Personnel Justification

Marcia N. Gordon, PhD, P.I. [redacted] Dr. Gordon will oversee all aspects of the subcontract including intellectual direction, experimental design, USF personnel supervision, animal treatments, data collection, data analysis, and preparation of manuscripts. She will also be responsible for the financial oversight of the subproject. In particular, Dr. Gordon will be responsible for supervising all animal husbandry, including breeding, genotyping, assigning mice to experiments, administering therapeutics, behavioral assessments, cognitive testing and measurement of tauopathy. She is an established investigator with extensive knowledge in neuroscience and has been studying neuroinflammation in Alzheimer's disease for over 15 years. Salary support plus university required fringe benefit and health insurance rates are requested. Salary requests are raised by 2% for the second budget period.

Dave Morgan, PhD, Co-I. [redacted] Dr Morgan has directed a basic science research program on questions related to aging brain and Alzheimer's disease for 30 years. He has also led a team of investigators studying murine models of both amyloid and tau pathology for the last 20 years. Dr. Morgan's research focus has been on therapeutics with emphasis on the role of CNS inflammation. He will assist with developing study design, data analysis and interpretation, and manuscript preparation.

[redacted] technician [redacted] will be responsible for performing the experiments described in this application, including administration of agents to mice, performing behavioral and cognitive testing of mice, and assessing levels of acetylated proteins by Western blots. Ms. Petty has been employed by the PI for approximately 1 year, and is fully trained with the necessary skill set to perform these experiments.

Laboratory Supplies. Funds in the amount of [redacted] are requested to allow purchase of general purpose chemicals and other lab supplies necessary for the completion of this project. This may include antibodies for immunostaining and Western blot analyses, supplies for genotyping mice, personal protective equipment, laboratory disposables, and other supplies.

Mouse Costs. Funds are requested to generate and maintain sufficient numbers of transgenic mice for this project which are not available elsewhere. At current per diem rates of $0.92, maintaining 32 mice until 5 month of age (treatment from 2.5-5 months x 2 drug conditions) and 64 mice until 7 months of age (drug vs vehicle treatment from 5-7 and 2.5-7 months) will require [redacted], respectively. Additional funds are requested to maintain breeding stock of the background strains and Tg4510 mice at USF. The requested costs are based on our past experience with generating these lines for experiments of similar size to those proposed here.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)
Section A, Senior/Key Person	[redacted]
Section B, Other Personnel	[redacted]
Total Number Other Personnel	[redacted]
Total Salary, Wages and Fringe Benefits (A+B)	[redacted]
Section C, Equipment	
Section D, Travel	
1. Domestic	
2. Foreign	
Section E, Participant/Trainee Support Costs	
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
6. Number of Participants/Trainees	
Section F, Other Direct Costs	[redacted]
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other 1	[redacted]
9. Other 2	
10. Other 3	
Section G, Direct Costs (A thru F)	[redacted]
Section H, Indirect Costs	[redacted]
Section I, Total Direct and Indirect Costs (G + H)	[redacted]
Section J, Fee	

Tracking Number: GRANT12375369 Funding Opportunity Number: PAS-17-065 . Received Date: 2017-03-31T12:25:30.000-04:00

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	[redacted]	0	0	0	0	[redacted]

OMB Number: 4040-0001
Expiration date: 06/30/2016

SBIR/STTR Information

Program Type (select only one)*

○ SBIR ● STTR ○ Both (See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)

SBIR/STTR Type (select only one)*

● Phase I ○ Phase II ○ Fast-Track (See agency-specific instructions to determine whether a particular agency participates in Fast-Track)

Questions 1-7 must be completed by all SBIR and STTR Applicants:

1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?* ● Yes ○ No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* 2

2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* ○ Yes ● No

If yes, insert the names of the Federal laboratories/agencies:*

3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov * ○ Yes ● No

4. Will all research and development on the project be performed in its entirety in the United States?* ● Yes ○ No

If no, provide an explanation in an attached file. Explanation:*

5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work?* ○ Yes ● No

If yes, insert the names of the other Federal agencies:*

6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?* ○ Yes ● No

7. Commercialization Plan: If you are submitting a Phase II or Phase I/Phase II Fast-Track Application, include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:*

Funding Opportunity Number: PAS-17-065. Received Date: 2017-03-31T12:25:30.000-04:00

OMB Number: 4040-0001
Expiration date: 06/30/2016

SBIR/STTR Information

SBIR-Specific Questions:

Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.

8.Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment.* ❍ Yes ❍ No

Attach File:*

9.Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?* ❍ Yes ❍ No

STTR-Specific Questions:

Questions 10 and 11 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 and 11 blank.

10. Please indicate whether the answer to BOTH of the following questions is TRUE:* ● Yes ❍ No

1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND

2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?

11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?* ● Yes ❍ No

Funding Opportunity Number: PAS-17-065. Received Date: 2017-03-31T12:25:30.000-04:00

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OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ❍ Yes ● No

*Agency-Defined Phase III Clinical Trial? ❍ Yes ❍ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ❍ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ❍ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

❍ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

Tracking Number: GRANT12375369

Funding Opportunity Number: PAS-17-065 . Received Date: 2017-03-31T12:25:30.000-04:00

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4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

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Study of the New HDAC6i SW-100 as a Treatment for Alzheimer's Disease and Other Tauopathies

Tauopathies are neurodegenerative disorders for which no effective treatments are known. Some disorders are caused by mutations in tau that appear to increase the probability of tau aggregate formation, leading to intracellular neurofibrillary tangles [1]. These are typically referred to as fronto-temporal dementias. Other disorders occur in other brain regions (corticobasal syndrome, progressive supranuclear palsy etc. [2]). The most common disorder to include tau inclusions is Alzheimer's disease, where the tau pathology correlates better than amyloid pathology with cognitive impairment [3]. Amyloid is argued to be the initiating factor in the disease and to increase tau pathology [4]. Recently it has been hypothesized that some of the deficits found in Alzheimer's models result from soluble forms of tau which disrupt dendritic function [5] rather than the intracellular fibrils.

Histone deacetylases (HDACs) are a family of proteins which remove acetyl moieties attached covalently to lysine residues in proteins. In the nucleus, HDACs generally promote chromatin condensation and repress gene expression. In transformed cells, these enzymes are thought to suppress proapoptotic programs, leading to unregulated proliferation. HDAC inhibitors are widely explored as treatments for cancer [6]. There are at least 18 isoenzymes in the HDAC family divided into 4 homology classes. Classes I, II and IV are Zn dependent, while class III, also known as sirtuins, are NAD dependent for their enzyme activity. Class I HDACs (HDAC1, 2, 3 and 8) are nuclear enzymes and are the major focus of research for anti-tumor agents. Class II enzymes are often tissue specific and divided into Class IIa (HDAC4, 5, 7, 9) enzymes which shuttle between cytoplasmic and nuclear compartments and Class IIb (HDAC6 and 10) which are primarily cytoplasmic and deacetylate nonhistone proteins. HDAC6 has been shown to traditionally act upon tubulin, cortactin and HSP90. Tubulin acetylation is associated with increased microtubule stabilization and axonal transport

Our team has pioneered the design and synthesis of a number of HDAC6 inhibitors with his isoform selectivity. In our prior work we tested our HDAC6i Tubastatin A (**TA**) in the Tg4510 mouse [7]. This was found to have nM potency in inhibiting HDAC6, but μM or greater concentrations were needed to inhibit most other HDACs (>1000x selectivity for all but HDAC 8 at 50 fold selectivity; Butler et al, 2010). Moreover, when tested in cells, this agent was found to increase the acetylation of tubulin, but not histone proteins [8]. This agent has been shown to reduce the phenotype in Charcot-Marie-Tooth disease [9]. This disorder is caused by mutations in the 27 kD small heat shock protein HSBP1, leading to decreased tubulin acetylation and axonal loss in peripheral neurons. **TA** treatment reversed both the loss of acetylated tubulin and the axonopathy in this model. **TA** has further shown benefit in increasing BDNF trafficking in neurons from models of Rett syndrome [10]. Tubastatin increased autophagy in neurons expressing mutant Huntingtin protein and diminished its accumulation [11]. Tubastatin improved outcomes in a rat stroke model [12]. Other HDAC6 inhibitors with greater CNS penetration also showed antidepressant activity in murine models of depression [13]. In the periphery, multiple models of inflammation benefit from **TA** administration including arthritis models, sepsis models, endotoxin induced pulmonary edema and asthma [14]. Thus HDAC6 inhibitors may have applications in many conditions both in the CNS and the periphery.

In prior work, we demonstrated that **TA**, a selective but modestly CNS penetrant HDAC6 inhibitor partially prevented the development of the tau phenotype in the aggressive Tg4510 model when treated between 5 and 7 mo of age (Selenica et al, 2014). **SW-100** is a new HDAC6i with selectivity similar to that of Tubastatin, but increased CNS penetration. **SW-100** further lacks mutagenicity in the Ames test (in which **TA** was positive). Thus, we wish to evaluate if this compound, as well as a back-up analog, can more fully reverse the phenotype of the Tg4510 mouse by pursuing the three aims below:

Aim 1. Prepare 4 new analogs of **SW-100** as potential back-up compounds, and conduct HDAC isozyme testing, tubulin acetylation assays, and ADMET assays. Advance the best of these to animal studies in Aim 2.

Aim 2. Conduct a dose range finding study of **SW-100** and the best back-up compound from Aim 1 to identify a dose in mouse chow that causes maximal CNS impact and is well tolerated. Mice will be treated for one week. Efficacy will be established by assaying for tubulin acetylation in the brain, and preliminary safety by observing for any side-effects.

Aim 3. Test **SW-100** and the back-up analog from Aim 1 in Tg4510 mice starting at two ages to ascertain the extent to which these NCEs can retard the development of the tau phenotype, and whether benefits can be observed even after tau deposition has started. Assessments will thus be made of drug effects on cognition, histological tau deposition, and neurochemical tau accumulation. Any positive effects observed using these drugs after tau deposition would suggest benefit for people who already have dementia.

Success in these three aims will further elevate HDAC6 as a viable drug target in tauopathy and provide support for the further development of **SW-100** and analogs for use in the human population. Advanced work would be conducted by application for Phase II funding.

A. SIGNIFICANCE

We initiated testing of Tubastatin in our Tg4510 model based upon its ability to stabilize microtubules, and enhance axonal transport [15]. However, since initiating these studies, several additional mechanisms by which HDAC6 inhibition may benefit tauopathy have been demonstrated. Cook et al, [16] showed that through acetylation of HSP90, HDAC6 depletion or inhibition increases the degradation of tau in cell models. Elevation of HDAC6 in cells promotes accumulation of tau. Thus HDAC6 inhibition will bias the actions of HSP90 towards degradation of its client proteins, reducing the levels of tau. A third potential mechanism is by regulating acetylation of tau itself. Tau is acetylated within the microtubule binding domain at lysines 269, 290, 321 and 353 [17]. Acetylation of these domains appears to be reciprocally related to the phosophorylation of adjacent serines, including ser 262 which is thought to signal pathogenic tau. Acetylated tau does not form fibrils[18] (although oligodendrocyte tau may respond differently[17c]).

HDAC6 is elevated in Alzheimer's disease tissue and acetylated tubulin is diminished [17b, 18-19]. Similar results are found in APPPS1-21 mice that deposit Aβ [20]. Moreover, HDAC6 is phosphorylated by several kinases, including GSK-3β which further increases HDAC6 activity [21]. There is some evidence that the toxicity of Aβ may be mediated in part through HDAC6. Kim et al [22] demonstrated that Aβ induced disruption of mitochondrial axonal transport was reversed by HDAC6 inhibitors. Yu et al, [23] demonstrated that memory deficits caused by intrahippocampal injections of Aβ were prevented by HDAC6 inhibition. Further, when APPPS1 mice were crossed onto an HDAC6 knockout background, the learning and memory impairments were prevented and tubulin acetylation was restored to normal levels [20a]. However, there were no changes in Aβ deposition. These effects are similar to those found when APP mice were crossed onto a tau knockout background [24], suggesting that increased HDAC6 might be one means by which Aβ can influence tau toxicity within neurons.

B. INNOVATION. There are presently no meaningful treatments for tauopathies. Although HDACs have been considered as therapeutic targets for Alzheimer's disease, most discussion (with little data) has centered upon their epigenetic regulation of gene expression through histone acetylation [25]. Our prior work has determined Tubastatin A (**TA**), a highly selective HDAC6 inhibitor, was effective in lowering tau and improving the behavioral phenotype of the Tg4510 mouse model of amyloid deposition (Figures 1-4 in experimental design below) [7]. While significant, the suppression of the tau phenotype by this agent was partial. Likely this is due to that fact that **TA**, while a useful tool compound, does not possess ideal properties for a drug that is active in the brain as its measured brain PK is low. For **TA** the brain/plasma ratio is 0.2 indicating modest brain penetration. For the new analog **SW-100**, which is the compound which we plan to advance further through this SBIR grant, it is 2.5, indicating good uptake of the drug in the CNS. Another critical feature of Tubastatin A limiting its use in chronic studies is the genotoxicity of the agent in the Ames test. This widely used screen for carcinogens suggests that long term treatment with **TA** could increase risk for cancer. **SW-100** is Ames negative, and it shares a selectivity profile very similar to that of **TA** (see Table 1 below). Thus, these improved ADMET features suggest **SW-100** will be a better agent for CNS HDAC6 inhibition, and that it is likely to have improved efficacy in the tau mouse model. We believe that the work disclosed herein holds tremendous promise for a host of neurological disorders given the importance of HDAC6 to tubulin acetylation and in turn to mitochondrial transport, a fundamental problem associated with such disorders.

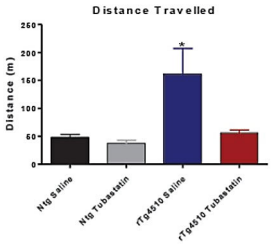


Figure 1. Rescue of rTg4510 hyperactivity by HDAC6 inhibition. Treatment of rTg4510 animals with **TA** significantly decreased the total distance travelled compared to the saline treated littermates (distance ± SEM, n=6) in open field test. Two way ANOVA revealed an overall effect of genotype ($p<0.026$) and treatment in the rTg4510 animal group ($p=0.03$).

C. EXPERIMENTAL DESIGN

Preliminary Data. The studies proposed below build on our initial findings with the selective HDAC6i, **TA** [7]. Tg4510 transgenic mice were bred by crossing two inbred lines, one with a P301L mutation in tau coupled to a tet response element and the other a line expressing Tet transactivator (tTA) driven by a CaM Kinase II promoter. Each of these parental lines is maintained separately on different inbred backgrounds. This cross results in F1 hybrid mice with both tau and tTA genes (designated Tg4510) that begin overexpressing tau postnatally in forebrain neurons. Non-transgenic littermates and tTA only mice of the same F1 background are also generated as control mice that do not develop tau overexpression and deposition. Addition of doxycycline will disrupt the tTA regulation of tau leading to suppression of transgene expression[26].

For studies with **TA**, we used 12 Tg4510 and 12 nontransgenic littermates. Half were injected i.p. once daily with 25 mg/kg Tubastatin in saline and the other half with saline vehicle (6 per group starting at 5 mo of age). During the final two weeks of the study mice were administered a 2 week behavioral test battery by an investigator unaware of the group identities of the mice. Mice were deeply anesthetized at 7 months of age with pentobarbital while placed on an isothermal pad to avoid cooling induced increases in tau phosphorylation, perfused with saline and the brain was removed. One hemisphere was immersed into paraformaldehyde for histological studies and the other was dissected into hippocampus, cortex and other brain regions for chemical studies. Sample numbers were coded so that investigators were blind with respect to treatment group identity. The Tg4510 mice have a behavioral phenotype that consists of hyperactivity and memory deficits, particularly in spatial navigation tasks. We found that **TA** treatment partially rescued this phenotype. In Figure 1, we show the activity of the mice in an open field chamber, assessed by total distance traveled.

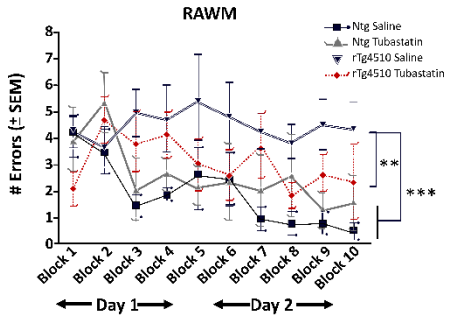


Figure 2. Rescue of rTg4510 memory impairment by HDAC6 inhibition. Number of errors made (average ± SEM) while finding the hidden platform in the RAWM for days 1 and 2. A significant memory impairment of rTg4510 was found compared to non-transgenic animals by 2 way ANOVA and Fisher's PLSD test (***, p<0.0001). **TA** treated rTg4510 mice performed better than vehicle treated littermates (p-0.002) and were not significantly different from the **TA** treated non-transgenic mice (p=0.3).

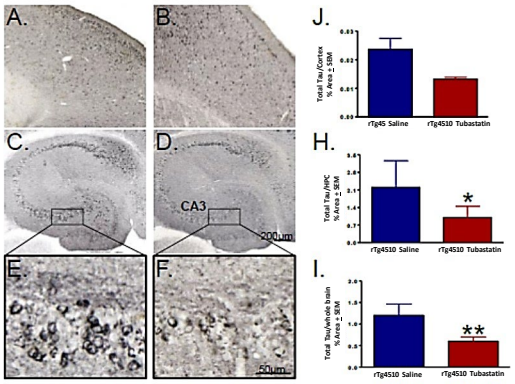


Figure 3. HDAC6 inhibition decreases total tau levels in the brain. Micrographs in panels A-F represent stain for total tau levels by immunohistochemistry. Reduction in tau levels were observed in cortex and hippocampus regions of Tubastatin rTg4510 mice (B, D, high magnification of CA3 shown in panel F) compared to saline treated littermates (A, C, high magnification of CA3 panel E). Percent of stained area and analyzed by Student's t test (J-I). (cortex p=0.055; hippocampus, *p<0.05; right hemisphere **p<0.001, n=6). Endogenous tau was not detectable in nontransgenic (ntg) mice and were thus excluded from the statistical analyses.

We find that the saline treated Tg4510 mice have the expected increase in open field activity, and that treatment with **TA** reverses this increase, with no impact upon the nontransgenic mice.

The radial arm water maze is a variant of the Morris maze that requires mice to use a spatial strategy to solve the task by imposing swim alleys onto the pool[27]. The saline treated Tg4510mice failed to learn the task and were significantly different from the non-transgenic mice. **TA** treatment significantly improved the performance of Tg4510 mice compared to saline treated Tg4510 mice (Fig 2). However, the treated mice did not reach the less than one error criterion typically observed in groups of mice that learn the task well. Thus we consider this a partial rescue. The histological changes in tau deposition are shown in Fig 3. Sections are imaged with a digital scanning microscope permitting measurement of entire regions. Using total tau antibody H150, we observed significant reductions of roughly 50% in total tau staining in hippocampus and whole brain (Figs 3H and I) and a similar trend in cerebral cortex (4G). Representative micrographs are shown in panels A-F. Surprisingly, none of the phospho forms of tau were decreased in these mice, nor were the number of silver stained deposits detected by the Gallyas method [7].

Neurochemical data support the histological findings. Using western blot analysis, we find a roughly 40% reduction in total tau, but no changes in the phospho forms of tau in brain homogenates (Fig 4). When we separate the homogenate into detergent soluble and insoluble fractions [28], we find little difference in the detergent soluble fraction (not shown), but large reductions in all forms of tau in the detergent insoluble (formic acid soluble) fraction (Fig 5).

Importantly, we also find that antibodies specific for acetylated tubulin show the expected increased signals in the mice that were treated with Tubastatin. This is true both when normalized to total tubulin and to GAPDH, indicating this is not secondary to an impact of the drug on tubulin expression. This confirms that drug was reaching the central nervous system and impacting the intended target.

These studies show that: a) **TA** does impact acetylation of tubulin in the CNS, suggesting the dose administered is adequate to hit the target; b) the Tg4510 behavioral phenotype is at least partially reversed by 2 months of treatment and c) this is associated with a reduction in total tau (but not the phosphorylated forms of tau). Questions to be addressed below include a) would a next generation HDAC6 inhibitor with superior brain penetration to TA have greater impact on the tau phenotype and b) would earlier treatment arrest the progression of phospho-tau deposition as well as lower total tau levels? *If the improved more drug-like HDAC6i show more favorable outcomes, this would become a likely candidate for clinical study.*

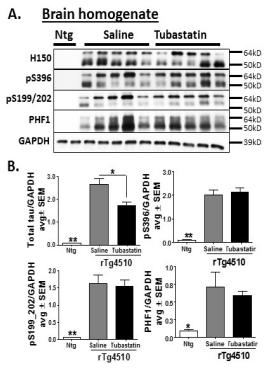


Figure 4. TA treatment reduced total tau in the detergent soluble fraction. Representative blots are shown at the top of the image, with relevant lanes labeled by treatment condition (top), tau isoform (right), and molecular weight (left). Lower panels depict quantitation. All isoforms of tau were present in higher amounts in Tg4510 mice vs nontransgenic (Ntg) littermates (* p<0.05, ** p<0.01). Total tau assessed with H150 antibody was significantly reduced after TA treatment.

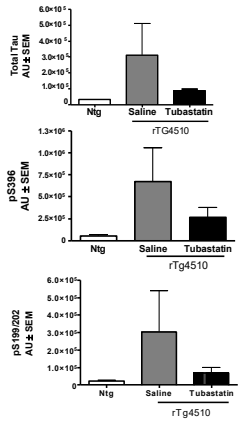


Figure 5. Decreased tau in deter-gent insoluble fraction with **TA** treatment.

Preliminary Chemistry. The development of HDAC class/isoform selective inhibitors has long been a goal of researchers and pharmaceutical companies, but due to the high sequence homology among HDAC isoforms, only a handful of truly selective inhibitors have been reported. We wish to underscore the fact that we have made and screened several hundred HDACis, and that the most promising of these compounds have proven to be selective for HDAC6[29] Based on its potency, selectivity, and ease of synthesis, **TA** remains one of the best-in-class, at least in terms of being a chemical tool compound [29]. The older, more structurally complex HDAC6i Tubacin is problematic to use due both to its lengthy synthesis and its poor solubility. The selectivity of both of

these compounds for HDAC6 is apparent from our data in Table 1.

Table 1. Table 1. Enzyme Inhibition Data for Tubacin, Tubastatin A and SW-100 at all 11 HDAC Isozymes[a].

	Tubacin (IC_{50}, μM)	Tubastatin A (IC_{50}, μM)	SW-100 (IC_{50},
HDAC1	1.40	16.4	5.22
HDAC2	6.27	>30	>30
HDAC3	1.27	>30	>30
HDAC4	17.3	>30	11.0
HDAC5	3.35	22.9	4.55
HDAC6	**0.004**	**0.015**	**0.003**
HDAC7	9.7	>30	4.07
HDAC8	1.27	0.814	3.34
HDAC9	4.31	>30	3.46
HDAC10	3.71	>30	>30
HDAC11	3.79	>30	0.746

[a]IC_{50} data (μM); of compounds tested in duplicate in 10-dose IC_{50} mode with 3-fold serial dilution starting from 30 μM solutions. IC_{50} values were extracted by curve-fitting dose/response slopes.

Table 2. Preliminary HDAC and ADMET screening of SW-100.

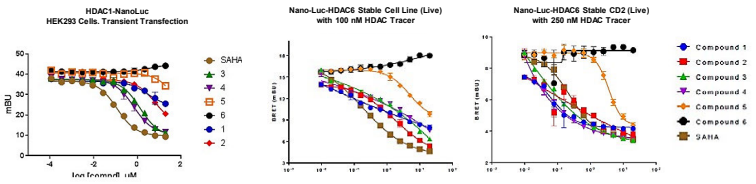
Liver microsomal stability ($t_{1/2}$ min, with NADPH)	Human	23
	Mouse	23
Hepatocyte stability (t1/2 min)	Human	30
	Mouse	10
hERG test	IC_{50}, µM	12
Mini-Ames test	TA98, TA1537	negative
CYP inhibition (%@10 µM)	1A2	21
	2C9	19
	2C19	83
	2D6	2.5
	3A4	-0.6
PK profile (oral administration, dose 100mg/kg)	$t_{1/2}$	2.5 hrs
	Cmax	500 ng/mL
	AUC_{inf}	783 hr·ng/mL
	Brain/plasma	2.5

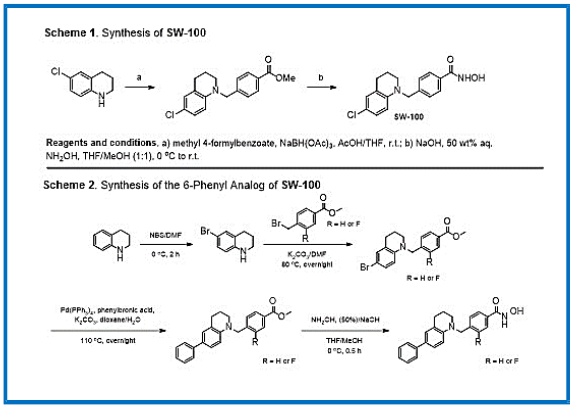
TA has been studied in a variety of in vitro and in vivo disease models. In particular, TA ***was not cytotoxic*** in a neuronal culture model, even at the highest concentrations tested (20 µM). In terms of ADMET, the ***brain penetrance of TA was found to be modest*** (AUC_{brain}/AUC_{plasma} = 0.18), and we estimate that at the doses being used in the RTT animals, we are achieving a brain concentration of ~150 nM. However, our findings that TA and some related analogs are positive in the Ames test have directed us to identify other hydroxamate-containing HDAC6is that lack Ames activity. We have succeeded in identifying such compounds. In particular our lead compound, **SW-100** (containing a tetrahydroquinoline (THQ) cap), showed excellent potency against HDAC6 and selectivity against HDAC1 and well as the other isoforms (Table 1). To investigate the possible mutagenicity of **SW-100** was incubated with two strains of *Salmonella typhimurium* (TA98 and TA1537) in the presence and absence of mammalian microsomal enzymes (S9 mix). The results showed that **SW-100** was negative under the conditions of this mini-Ames assay. **SW-100** was also evaluated in the liver microsomal and hepatocyte stability assay, as well as for hERG and CYP inhibition. PK studies showed that the drug is relatively well absorbed and is able to reach significant plasma concentrations upon oral exposure in test animals for a period of 2.5 hrs in spite of the measured short $T_{½}$ in liver microsomes and hepatocytes, a feature that we believe we can improve through proper formulation (to be contracted to a CRO). The data in Table 2 also show that inhibition of the hERG and CYPs are generally in an acceptable range. Additionally, **SW-100** is brain penetrable as demonstrated by the measured brain/plasma AUC ratio of 2.5. The method of synthesis of **SW-100** is provided in the chemistry section below. NanoBRET target engagement assays (Promega) were further carried out to confirm the intracellular potency and selectivity of several of the HDACi, using methodology that is based on the competitive displacement of a SAHA-linked fluorescent tracer instead of the usual acetyllysine substrate based methods used to assess activity against the isolated enzymes (Table 3). Interestingly, the results shown in Table 3 for **SW-100** are close to the isolated enzyme data reported in Table 1. **SW-100** was also investigated in N2a cells for assessment of the functional selectivity. The relative levels of tubulin/histone acetylation were measured. These data indicate that **SW-100** displays similar acetylated tubulin levels and acetylated histone levels with those found for our reference HDAC6i **TA** (data not show, ratio of acetylated tubulin/tubulin was 8.7 for **SW-100** when tested at 1 mM).

Table 3. HDAC1/6 NanoBRET target engagement (TE) assay for compound SW-100 and SW-101.[a]

COMPOUND	HDAC1	HDAC6	HDAC6-CD2
	(IC_{50},nM		
SW-100 (compound1)	3004	2.12	69
SAHA	0.1172	0.1849	0.2414

[a]The NanoBRET™ target engagement (TE) assay was carried out by Promega, WI.

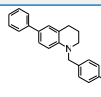
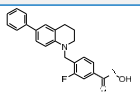
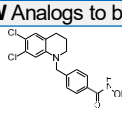
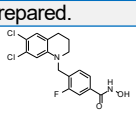




New Chemistry: The syntheses of the lead compound **SW-100** is shown in Scheme 1. **SW-100** was obtained in two steps starting from the chlorotetrahydroquinoline shown by reductive amination to form the desired ester and the reaction with NH_2OH to yield the hydroxamate. Based upon various physicochemical and ADMET calculations we propose to prepare four other **SW** analogs and to fully characterize these for HDAC selectivity and in vitro ADMET. The compound showing the best parameters will be selected as a backup compound to **SW-100** for study in the tau mouse model. Note that the LogBB values of three of these compounds are slightly

better than that of **SW-100**, while the other compound has a comparable LogBB. These compounds were selected in part based on modeling studies using the x-ray structure of HDAC6 from zebra fish, and we anticipate that these newer analogs may show improved potency because of the higher calculated, binding efficicies. Due to lack of space we are unable to expand on these modeling studies in this application. An exemplary synthesis of one of these new analogs can be found in Scheme 2.

Table 4. Structures and Properties of Related **SW** Analogs to be Prepared.

Property				
MW	358.44	376.43	351.23	369.22
clogP	4.74	5.08	4.39	4.73
tPSA	52.57	52.57	52.57	52.57
logBB	0.12	0.25	0.37	0.43
$logD_{7.4}$	3.91	4.02	3.99	4.02
Pka (base)	4.9	4.6	3.0	2.7
Pka (acid)	8.3	8.3	8.8	8.3

ADME/Tox Considerations. Rationale. Experimental ADMET studies will be performed on the four new analogs shown in Table 4 as long as they have an HDAC6 selectivity profile comparable to **SW-100**. The ADMET studies will allow us to better ensure that these compounds are suitable for animal testing and beyond. As always, it is best to obtain ADMET data at an early stage, to maximize our chances of identifying viable clinical candidates, while avoiding costly animal experiments on problematic drug candidates. The pharmacological data and ADMET results are used in concert to determine which compounds will be advanced further for study in the CMT2A animal model, and subsequently to additional safety profiling. We already have the required ADMET data on **SW-100**. We will also investigate any off-target effects of HDAC6 ligands selected for advancement. Thus, we will contract to Reaction Biology Corporation to screen against the sirtuins as well as available zinc metalloproteases including the MMPs. A complete Cerep screening profile will be conducted on these two candidates as well to ensure that there is no off-target activity. **Plan.** We will use appropriate CROs, and in particular Pharmaron for ADMET studies. Thus, we will scale up the **SW** compounds and submit them for all required ADMET work; the data required are outlined in Table 5. Thus metabolic stability, hERG activity, CYP450 induction/inhibition, plasma protein binding, Caco-2 permeability assay, assessment of P-glycoprotein substrate activity, brain and plasma pharmacokinetic profiling [$T_{1/2}$, C_{max}, AUC), overall bioavailability (%F), Ames testing as well as chromosomal aberration testing. In a Phase II application, it would be our plan to submit the most efficacious compounds from the animal studies to the Charles River for both acute and chronic toxicity studies including rat cardiotoxicity and pulmonary effects.

Table 5. Preclinical Attributes of HDAC6 Inhibitors

Property	Best	Acceptable
Dose	< 50 mg	< 500 mg
Dose frequency	1/day	bid
Potency and Selectivity for HDAC6	*< 50 nM, > 500X*	*< 75 nM, > 100X*
Cellular Activity	< 1uM	< 5uM
Plasma Protein Binding	< 95%	< 99%
Solubility	> 60 μg/mL	10 – 60 μg/mL
Cyps 3A4 and 2D6 inhibition	< 50% at 30 μM	< 50% at 10 μM
Microsomes: % compound remaining at 60 min	Human > 85%, rat > 70%	Human >75%, rat > 60%
Bioavailability rat	Rat > 20%	Rat > 10%
Metabolic half life, hours	> 1.5 h rat	> 1 h rat
Ames and micronucleus	Negative	Negative
hERG	Negative, IC_{50}/C_{max}, unbound > 30	Negative, IC_{50}/C_{max}, unbound > 30
Cerep Profile	No limiting pharmacology	No limiting pharmacology
Multiple dose tolerance	No issues at 10X expected dose	None at 5X expected dose
Caco-2 Permeability,	>20	10
Caco-2 efflux ratio	< 1	< 3

Aim 2. Dose ranging study of SW-100 administered to mice in food . *Rationale Aim 1.* Several groups have experience with SW-100 administered by IP injection. Single injections of 5 to 30 mg/kg have resulted in increased tubulin acetylation in sciatic nerve in a dose-related fashion (unpublished data from StarWise). The compound was administered 20 or 100 mg/kg acutely for 4 months without any apparent toxicity.

One of our end points in Aim 3 is the behavioral phenotype of the Tg4510 mice. Our experience with daily injections or gavage finds this can interfere with the performance of control and transgenic mice on some of the behavioral tasks we employ. Moreover, the SW-100 half-life would suggest two drug injections daily to maintain adequate exposure. However, as we do plan to have a CRO investigate a better drug formulation, it is likely that we will be able to reduce this to once per day. Nonetheless, to avoid these issues, we plan to evaluate the use of dietary administration of the agent through the chow. This has the advantage of being non-stressful and provides multiple administrations throughout the day as the mice normally feed. We have considerable experience with this administration method in past studies [30].

Experimental Design Aim 2. We will use 3 mo old nontransgenic littermate mice on the same F1 hybrid background as the Tg4510 animals for these studies. Mice will be singly housed and fed the mouse chow lacking any added drug for one week prior to starting the **SW-100**d-enriched diets. Food and water intake will be monitored by weighing water bottles and food pellets each day. Our experience is that a 30 g mouse consumes 5 g of food daily and 5 ml of water daily. We will calculate the amount of **SW-100** to compound with the mouse chow based on these averages, but will also calculate exact amounts based on food consumption measurements and mouse weights. We will offer mice chow (prepared by Dyets, Bethlehem PA) calculated to deliver 0, 10, 20, 40, 80 or 160 mg/kg/d of **SW-100**. Mice will be offered the chow for 7 days with daily measurements of amounts consumed. A modified Irwin test battery will be used to evaluate neurotoxicity [31]. Additionally, mice will be monitored daily for sickness behavior, by means of the method of Gandhi et al [32] using a) 2 min videotaped in cage observation each morning rated by 2 blinded investigators. After 7 days we will collect mouse tissues at three time points; 1700 hours (one hour prior to lights out), 1900 hours (one hour after lights out) and 0700 hours (one hour after lights on). Mice consume roughly half their daily ration of

food within the first hour of lights out. Thus we expect the maximum drug exposure will be captured at the 1900 hour time point. The minimum exposure will likely be at 1700 hours and an intermediate exposure at 0700 hours shortly after mice have started sleeping. Sample size will be 6 mice per group. For tissue collection, mice will be overdosed with IACUC approved veterinary anesthetic and samples of plasma, brain and liver will be collected. One portion of each sample will be shipped to by the biology group to StarWise for measurement of the drug content in these specimens. In addition, samples of brain and liver will be evaluated by western analysis for the content of acetylated tubulin and HSP90, as these are two known substrates of HDAC6. We will further measure histone acetylation to avoid concentrations that inhibit other HDACs. **Statistics**. The dose-response data will be evaluated by one way ANOVA followed by Fisher's means comparisons.

Expected Outcomes Aim 2. We expect that we will find a dose related increase in plasma **SW-100** at all time points and that the brain concentration will exceed the plasma concentration. We further expect that the acetylation of HDAC6 substrates will be elevated in a dose dependent manner. An important question will be whether the acetylation continues to increase to the highest dose, or whether there is a maximal increase in acetylation of HDAC targets at the 40 or even 20 mg/kg dose. We will use this information in our selection of the dose to choose for the chronic treatment of Tg4510 mice in Aim 2. Further, we will avoid doses that also elevate histone acetylation.

The other critical question regards appearance of sickness behavior in these mice. We expect that all of these doses will be well tolerated. HDAC6 null mice have no apparent phenotype [20a], implying that on-target effects of the drug are unlikely to cause severe biological defects. One potential problem is that mice reduce their food intake when first offered the drug enhanced diets. We have experience that on some occasions, the modified flavor of the diet induces a transient reduction in intake (consistent with bait-shyness behavior) that is subsequently compensated for by increased consumption on later days. We further do not expect to observe sickness behavior in these mice. If at the higher doses we observe sickness behaviors, we will exclude that dose from consideration in Aim 2. Note that one study has already administered 40 mg/kg **SW-100** in divided doses for 2.5 months without signs of toxicity. We will choose the dose that achieves the highest level of tubulin acetylation without increasing histone acetylation or inducing sickness behavior. We will further calculate the ratio of **SW-100** concentration to its IC_{50} for HDAC6 for all tissues.

Potential problems, Aim 2. One potential problem is that the drug enhanced diet has a taste that makes it unpalatable, and leads to reduced intake for the entire week. In the past we have used saccharin to mask bitter flavors in diets and would attempt this solution. Another potential problem may be that all doses induce sickness behavior. If that is the result we will add groups receiving 2 and 5 mg/kg/d of **SW-100** or backup analog to try and identify a tolerable dosage that still leads to increased brain tubulin and HSP90 acetylation. Stability of the drug may also be an issue although we believe this is unlikely given our current state of knowledge. We store the drug-enhanced mouse chow in a 4° cold room before dispensing. We will send a portion of the mouse chow to StarWise immediately after receiving the material from the compounding company to rigorously confirm proper compounding. We will leave one sample at rt for the duration of the study and send it with the tissue samples and refrigerated diet at the end of the study to confirm the stability of the compound in chow over time, even at room temperature.

Aim 3. Time course of TA effects on the phenotype of Tg45410 mice.

Rationale Aim 2. The Tg4510 mouse is an aggressive model of tauopathy, with deposits readily detectable by 3-4 mo and neuron loss evident as early as 6 mo [26, 33]. This aim will determine the overall efficacy of HDAC6 inhibition by **SW-100** on the tau phenotype. Our **TA** study started at 5 mo of age, a time when considerable tau pathology was already present. We achieved a partial reversal of both the behavioral and some of the histopathological phenotype with a two mo treatment. This Aim will test whether a) the more brain penetrant **SW-100** inhibitor is more efficacious and b) and if earlier initiation of therapy will produce a more complete inhibition of the Tg4510 phenotype.

Experimental Design Aim 2. This experimental design was developed using principles outlined in [34] to increase rigor in therapeutic trials using mouse models.Tg4510 mice will be started on **SW-100** in the chow (using the dose obtained in Aim 1) or control chow in three groups; 2.5 to 5 mo of age (before deposition); 2.5 to 7 mo, or 5-7 mo (after significant tau deposition). The control chow treated Tg4510 group collected at 5 mo will serve as baseline pathology for the 5-7 mo treatment group. Group size will be 16 mice balanced with respect to gender and litter and assigned randomly to treatment condition. Gender will be considered as a variable. We will also include groups of treated and untreated nontransgenic mice for inclusion in behavioral assays and measures other than tau deposition (histochemistry and immunoblots). All mice will be assigned a coded number such that investigators making subsequent measurements are blind to the group identity.

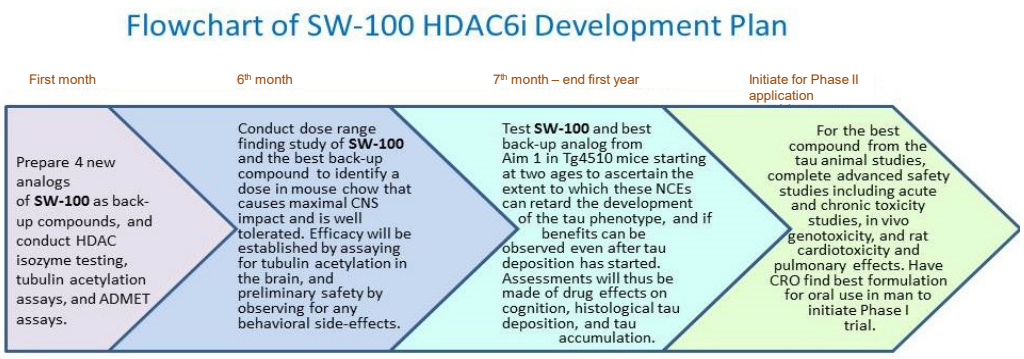
Mice will be tested in a two week behavioral test battery at the end of the treatment period including open field activity, rotarod performance, Y maze, radial arm water maze (2 day variant) and fear conditioning. Shortly after behavioral testing tissues will be collected for measurement of drug in brain and plasma, estimation of histopathology and dissection into brain regions for chemistry using our standard methods [35]. The left hemisphere used for histopathology will be immunostained for total tau, and three phosphorylated tau (p-tau) forms (p-199-202, p262 and p396), Gallyas silver stain, GFAP (to estimate astrocyte activation), Iba-1 (endogenous microglia), CD45 (activated microglia and infiltrating leukocytes including macrophages), CD68 (phagocytic microglia/macrophages), and MHC-II (antigen presenting microglia/macrophages). Stereologic estimation of regional volumes and neuron counts will be performed using Nissl stains. Methods, including number of sections (8 per mouse) will be as in [36]. Quantification will use a digital scanning microscope. From the right hemisphere, hippocampus and anterior cortex will be dissected, and homogenates will be used for western analysis of detergent soluble and insoluble tau using markers for total tau and three p-tau isoforms. We will separately quantify the high molecular weight tau bands that most likely represent oligomeric forms. We will also use these homogenates for measurement of tubulin acetylation, HSP90 acetylation

and tau acetylation to estimate the degree of HDAC6 inhibition [16]. We will further measure p62 and LC3 to estimate formation and degradation of autophagosomes, that have been reported to both increase or decrease with HDAC6 inhibition [11, 21]. The posterior cortex will be used for RNA preparation to estimate the levels of mRNA for the tau transgene (to rule out an effect of **SW-100** on transgene expression as an explanation for the results). **Statistics**. We have previously found that 16 mice are sufficient to detect changes of 20% or greater with 80% power in most of the dependent variables discussed here. We will perform a 2 way ANOVA at each time point for the behavioral measures and other measurements including nontransgenic control mice, with the factors being drug treatment and genotype. We will perform t-tests on the histopathological and chemical measurements for tau at each time point in only the Tg4510 mice, as there is no such pathology in the nontransgenic animals.

Expected Outcomes Aim 3. We expect that beginning the treatment early will have a greater impact on the tau-related phenotype of the Tg4510 mice than beginning the treatment after deposition has started. We certainly expect to see reductions in the behavioral deficits and in the total tau measures confirming the **TA** data. We also expect that a reduction in p-tau variants. At the 7 mo time point, we will expect to find rescue from the neuron loss with mice started at both 2.5 and 5 mo, and less hippocampal atrophy (as we detected for fractalkine treatment ;[37]). When compared to the baseline values at the start of the 5-7 mo treatment (tissue collected from control chow treated 2.5-5 mo old mice), we expect that the Tg4510 mice treated from 5-7 mo will show little increase in pathology. One possible but surprising outcome would be a reduction in tau pathology in SW mice treated from 5-7 mo compared to vehicle treated Tg4510 mice collected at 5 mo. This would imply the potential for HDAC6 inhibition to clear pre-existing tau deposits. Finally, we expect that the overall effects of **SW-100** on reducing the tau phenotype in the 5-7 mo group will be more complete than the effects of TA over the same age range. In general, we anticipate that the glial histological markers will correlate with the tau pathology, as we observed previously [38]. We expect reduced microglial activation will be correlated with a rescue of the neuron loss and brain atrophy found in these Tg4510 mice. The CD45 marker is argued to indicate highly activated cells that may have recently infiltrated from the periphery. CD68 is a marker for phagocytic activity, while MHC-II is a marker for antigen presentation. Iba-1 is a generic marker of all brain derived microglia, and general activation. Differential changes in these glial markers will be interpreted according to these proscribed functions[39]. A key question is whether these agents can stop the relentless neurodegeneration found in these mice. We will measure this in hippocampus by estimating neuron numbers in the CA1 and dentate gyrus regions of hippocampus using stereology. As part of this measurement, we will also estimate hippocampal volume and total cortical volume and compare these values to those from nontransgenic mice as an index of atrophy (hippocampal atrophy at 6 months is 30-40%[37]. We will also measure brain weights at tissue collection. We anticipate neurodegeneration will be reduced by **SW-100** treatment, compared to the untreated nontransgenic littermates.

Potential Problems Aim 3. One potential problem could be that the **SW-100** benefits the tau mice behaviorally, but slightly impairs the nontransgenic mice. This would suggest considering a lower dose in subsequent studies that does not impair behavior in nontransgenic mice. We do not expect this given our preliminary data showing no impairment in **TA** treated nontransgenics, nor is behavior reported to be impaired in HDAC6 knockout mice [20a]. A second possibility is that the dose selected may interfere with the expression of the transgene (which is unlikely since **SW-100** has little impact on histone acetylation except at extremely high doses), and artifactually reduce the tau accumulation. We will measure htau mRNA which we do not expect to change. If **SW-100** does reduce the htau mRNA, this will require run-on transcription studies to determine if the reduction is at the level of RNA transcription, or stability. If the reduction is RNA stability, this will also be examined for mouse tau RNA in non-transgenic mice. Reducing tau RNA may constitute yet another mechanism by which HDAC6 inhibition might slow tau accumulation. However, if **SW-100** causes a selective reduction in the human transgene RNA (possibly due to interference with the tet-transactivator) and not the mouse tau RNA, this would necessitate trying a lower dose of **SW-100**.

Determination of SW-100 concentration in plasma and tissue samples by LC/MS.



Plasma samples will be collected after anesthesia by cardiac puncture Brain samples will be collected following saline perfusion, quick frozen in an ice box and kept at -80 °C. All brain samples will be weighed and homogenized with water employing a brain weight (g) to water volume (mL) at a ratio of 1:3 before analysis. Internal standard will be added (JB-7-2, a brain accessible analog of **SW-100**). Protein will be precipitated in acetonitrile. The actual concentration is the detected value multiplied by the dilution factor. Concentrations of test article in the plasma and brain samples will be analyzed using an LC-MS/MS method. WinNonlin (PhoenixTM, version 6.1)

Time line. Aim 1 will be started and largely completed in the first half of year 1. Aim 2 will start after the first 6 months. Aim 3 treatment of the mice with **SW-100** and the best back-up compound would start in month 7 and finish in 7 months time in order to allow time for data collection.

G. VERTEBRATE ANIMALS

G1. Description of Procedures. All animal manipulations will be performed at the University of South Florida in an AALAC-approved vivarium facility using guidelines described in the "Guide for the Care and Use of Laboratory Animals."The goal of this project is to evaluate a specific therapeutic to prevent accumulation of abnormal tau variants, neuropathology, neurodegeneration, and behavioral impairments in a mouse (*Mus musculus*) model with overexpression of tau (rTg4510). The small molecule therapeutic will be administered in rodent chow, an oral administration route with less stress than repeated gavage. In Experiment 1, a range of doses will be administered for 7d, mice will be euthanized at specific times of the circadian rhythm in conjunction with feeding behavior, and drug levels will be measured in plasma, brain and peripheral tissues. Functional assays will include assessment of acetylation ratios of tubulin, HSP90 and histone3. This experiment will determine a drug dose that provides adequate delivery via chow. For each analog tested, this experiment will utilize 6 doses x 3 euthanasia time points x 6 mice per treatment condition = 108 nontransgenic mice of the same genetic lineage as the experimental mice (see below). In Experiment 2, rTg4510 mice or nontransgenic littermates will receive control or drug-containing chow from either 2.5 to 5 mo of age (prevention strategy), 5-7 mo of age (therapeutic strategy) or 2.5 to 7 mo of age. The group size will be 16 mice, balanced to include equal numbers of males and females, because both men and women are affected with Alzheimer's disease and other tauopathies. This experiment will use 2 genotypes x 2 drug conditions x 3 treatment durations x 16 mice = 192 mice. One-half will be rTg4510 with tauopathy and ½ will be nontransgenic littermates. Thus, testing each analog in this project will use 96 rTg4510 mice plus 204 nontransgenic mice that are generated in the same litters. Additional mice will be needed to breed to generate the rTg4510 mice and to maintain the background strains and transgenes.

G2. Justifications. This research project will employ mice that are genetically modified to develop brain pathology similar to Alzheimer and other tauopathy patients. This work must be performed in animals because it involves the understanding of cellular interactions important for the development of higher mental functions, such as learning and memory. There are no non-animal alternatives capable of displaying these features. There are no less sentient species that develop the brain pathology characteristic of Alzheimer's disease. Computational and in vitro studies have been completed to document target engagement and initial safety screening for this compound. This project will provide necessary proof of efficacy in a relevant animal model for future translation to human trials.

There are many reasons for selecting the rTg4510 mouse model for use in this project as opposed to other tau transgenic models. The rTg4510 mouse strain carries a transgene encoding a specific mutation in the MAPT (tau) gene (P301L) linked to human neurodegenerative disease in a cassette to allow regulated expression (tet-off). The rTg4510 mice are generated by breeding together a line of mice expressing the tau transgene under the control of the tet operon promoter [FVB-Tg(TRE-P301L tau] with a line of mice expressing the tet transactivator protein under the control of the Ca2+/calmodulin-dependent protein kinase (CAMK2) promoter [129S-Tg(Camk2-tTA)1Mmay/DboJ]. In mice with both transgenes (rTg4510), the tet transactivator protein is produced in neurons of the forebrain. The tet transactivtor protein binds to the promoter found on the tau transgene and allows its expression. We and others have demonstrated that high levels of tau over-expression in forebrain ensue, resulting in accumumulation of phosphorylated tau, formation of intracellular argyrophilic aggregations, synaptic and neuronal atrophy, and impairments in cognitive performance in spatial memory tasks such as the radial arm water maze. Because tau is selectively over-expressed in forebrain rather than the spinal cord, rTg4510 mice do not develop motor or spinal difficulties as other tau transgenic mice do. The rTg4510 mice develop tauopathy at a relatively young age compared with other tau mouse models (eg hTau), and have a faster rate of progression. This is an advantage for drug studies because it allows for a shorter duration of administration to observe effects on tauopathy. Finally, rTg4510 mice are an F1 hybrid, ensuring robust health (hybrid vigor) and minimizing strain-dependent pathologies. F1 mice are isogenic with genetic consistency, resulting is less phenotypic variation.

G3. Minimization of Pain and Distress. Discomfort, distress, pain, and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. No procedures associated with this proposal will require analgesia, anesthesia or sedation. The administration of drug via chow is associated with considerably

less stress and/or distress relative to repeated gavage or injection. The drug treatment is not expected to cause adverse events requiring palliative care or humane endpoints.

G4. Euthanasia. Methods consistent with the recommendations of the American Veterinary Medical Association Guidelines for the Euthanasia of Animals will be utilized.

MULTIPLE PD/PI LEADERSHIP PLAN

Because the chemistry and biology components of the proposed studies require specialized expertise, we have proposed a team approach, leveraging a group of highly qualified individuals from StarWise Therapeutics LLC and at the University of South Florida to jointly undertake this complex translational research study. This collaborative team consists of the contact PI (Dr. Alan Kozikowski, CEO) with expertise in modeling, chemical synthesis, and medicinal chemistry, and Drs. Marcia Gordon and David Morgan with extensive expertise in Alzheimer's disease cellular biology and AD mouse models. The USF team will study the effects of the new drugs being developed at StarWise Therapeutics to enhance tubulin acetylation, increase mitochondrial transport, and thus improve performance in the tau mouse model. This team has worked together for a number of years and has published together. They will conduct monthly Skype conferences and communicate on a regular basis by email and phone. All chemical samples will be shipped to USF from StarWise by Federal Express mail. If during the research program a potential conflict does develop, the PIs shall meet and attempt to resolve any dispute. If they fail to resolve the dispute, the disagreement shall be referred to an arbitration committee consisting of one impartial senior administrator from each of the PI's institutions and a third impartial senior administrator mutually agreed upon by all PIs. No members of the arbitration committee will be directly involved in the research grant or disagreement. Additionally, in accordance with NIH policy, any changes in key personnel will be submitted to the NIH program officer for approval of a replacement.

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CONSORTIUM/ CONTRACTUAL ARRANGEMENTS

Dr. Marcia Gordon at the University of South Florida (USF), Professor of Molecular Pharmacology and Physiology, is an expert in Alzheimer's tau mouse model behavioral studies. She and Dr. Kozikowski, as well as Dr. David Morgan, have been collaborating for some time on the possible use of various HDAC6 inhibitors (HDAC6i) in the treatment of Alzheimer's disease. They have formed an ongoing partnership to identify more effective HDAC6i as AD therapeutics. As the Kozikowski medicinal chemistry group possesses expertise in the design and development of HDAC6i, this partnership with USF is essential to advancing these new chemical entities to the clinic, as the USF team has all the required tools and experience in AD drug testing in appropriate animal models. The appropriate programmatic and administrative personnel of each organization involved in this grant application are aware of the NIH consortium agreement policy and are prepared to establish the necessary inter-organizational agreement(s) consistent with that policy.

RESOURCE SHARING PLANS.

Our IP policy, to guide sharing of intellectual property, and to regulate publication and distribution of program-generated materials, will follow NIH Guidelines. Data and materials generated in the course of the funded research will be made available to the research community. Information about materials that arise in the course of the funded research will be disseminated through timely peer-reviewed publications, seminars, and presentations at scientific meetings in accordance with the NIH Grants Policy Statement and the Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources: Final Notice, Dec. 1999. Our institutions firmly support the principal notion that technology arising from NIH-funded research should remain available and accessible to the research community. When licensing technology covered by pending patent applications or issued patents, such agreements include a reservation of rights provision, which ensures that such technology can be used both by our institutions and by third parties for educational and academic research purposes.

Findings from this research will be published in a timely manner, and deposited in the NIH manuscript repository whenever possible, to allow rapid dissemination of new knowledge. We will adhere to the NIH Grant Policy on Sharing of Unique Research Resources including the Sharing of Biomedical Research Resources Principles and Guidelines for Recipients of NIH Grants and Contracts issued in December 1999. We will assume responsibility for distributing any materials, and we will fill requests in a timely fashion. We will provide relevant protocols and published genetic and phenotypic data upon request. Material transfers will be made in accordance with the Uniform Biological Materials Transfer Agreement and without reach-through requirements. Should intellectual property issues arise, which require a patent, we will ensure that the technology remains available in accordance with the NIH Principles and Guidelines, however, it will be our policy to seek IP protection in a timely manner, to allow for eventual publication of the results.

Authentication of Key Biological and/or Chemical Resources

New Synthesized compounds:

All of the newly synthesized HDACi compounds that will be used in the proposed studies will be prepared by the Kozikowski group. All new chemical agents (NCEs) are routinely characterized by proton and carbon NMR, mass spectroscopy, elemental analysis, and HPLC. Well characterized control compounds such as Nexturastat and Tubastatin are used in the biology studies, thus allowing us to properly gauge the efficacy of the new compounds. Moreover, the spectral and HPLC data of all compounds to be submitted to Dr. Gordon are checked by a senior associate before they are allowed to go out, and must be of at least 98% purity. As such, the authenticity of all NCEs is properly controlled to allow for reproducibility. Any commercially available drugs used in these studies will also be checked by NMR as needed. In terms of the ADMET data obtained from the CROs, again control compounds are employed in all of these assays thus allowing for some quality assurance checks on the data received from them. In most cases, we will carry out our own microsomal stability studies, but these too will incorporate well known control compounds. If any of the ADMET data are found to be questionable or unexpected, we would then send the compounds to a second CRO to ensure data quality.

Animal Models:

Recombinant Tg4510 mice will be bred at USF as the F1 hybrid of FVB-Tg(TRE-P301L tau) and 129STg (Camk2a-tTA)1Mmay/DboJ. To minimize genetic drift, nontransgenic individuals of the parental strains will be purchased routinely and incorporated into the colony. All mice will be genotyped using established PCR reactions to ensure correct breeding and maintenance of the transgenes. Relative copy number will be monitored to minimize phenotype dilution. These are routine procedures in my laboratory. SW-100 will be provided by StarWise Therapeutics and subject to their internal quality control. LC-MS/MS will be used to measure drug levels in feed and biological samples. Compounded chow will be analyzed immediately after preparation and after storage mimicking normal use. Antibodies will be purchased from commercial suppliers for the characterization of proteins in immunohistochemistry (IHC) and Western analysis. Key antibodies, such as the anti-tau antibodies, will be validated using Western analysis with positive and negative control tissue samples. IHC antibodies will also be validated in each study using positive and negative control tissue sections; an example would be nontransgenic control animals and untreated rTg4510 (tau animal model) as negative and positive controls, respectively, for anti-phospho-tau staining. Antibodies specific for cell specific proteins, e.g. the immune marker CD45, can be tested using a cell line that is known to express this protein compared to a cell line that does not.