AWARD NUMBER: W81XWH-18-1-0696

TITLE:

Validating UBC9 as a Molecular Target to Develop a Therapy for All Forms of ALS

PRINCIPAL INVESTIGATOR: Steve Finkbeiner, MD, PhD

CONTRACTING ORGANIZATION: The J. David Gladstone Institutes

REPORT DATE: Oct 2019

TYPE OF REPORT: Annual Technical Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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Validating UBC9 as a Molecular Target to Develop a Therapy for All Forms of ALS

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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

J.DAVID GLADSTONE INSTITUTES
1650 OWENS ST
SAN FRANCISCO CA 94158-2261

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our goal is to identify common mechanisms involved in neurodegeneration in ALS and to use that information to identify molecular targets to discover and develop a new class of therapeutics that block disease progression of all ALS patients. We identified a novel enzyme Ube2i (UBC9) that may be critical for mediating degeneration of motor neurons (MN) caused by different genes and proteins that are linked to both familial ALS (fALS) and sporadic ALS (sALS) pathogenesis. Since UBC9 is an enzyme, small molecule drugs designed to inhibit it could be effective in treating all forms of ALS. Our goal here is to evaluate UBC9 knockdown in cellular models of ALS for neuroprotective properties.

15. SUBJECT TERMS

UBC9, ALS, TDP43, PFN1, TUBA4A, cytoskeleton, UBbe2i

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
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Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Our goal is to identify common mechanisms involved in neurodegeneration in ALS and to use that information to identify molecular targets to discover and develop a new class of therapeutics that block disease progression of all ALS patients. We identified a novel enzyme Ube2i (UBC9) that may be critical for mediating degeneration of motor neurons (MN) caused by different genes and proteins that are linked to both familial ALS (fALS) and sporadic ALS (sALS) pathogenesis. Since UBC9 is an enzyme, small molecule drugs designed to inhibit it could be effective in treating all forms of ALS. Our goal here is to evaluate UBC9 knockdown in cellular models of ALS for neuroprotective properties.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

UBC9, ALS, TDP43, PFN1, TUBA4A, cytoskeleton, UBbe2i

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Aim 1. Validate the role of UBC9 in neurodegeneration in different ALS cellular models.

Aim 2. Validate the role of UBC9 in neurodegeneration in vivo in murine models of ALS.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met.

Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Aim 1. Validate the role of UBC9 in neurodegeneration in different ALS cellular models.

We evaluated Ube2i (a.k.a. UBC9) knockdown in primary rodent neurons expressing either wildtype of mutant forms of ALS related genes. This was partly to confirm our previous findings and also to optimize the correct concentration of siRNA in our experiments as well as test against genes we had not tested before (i.e. FUS) (Figure 1). (Rodent neuron cultures were prepared using separate funds).

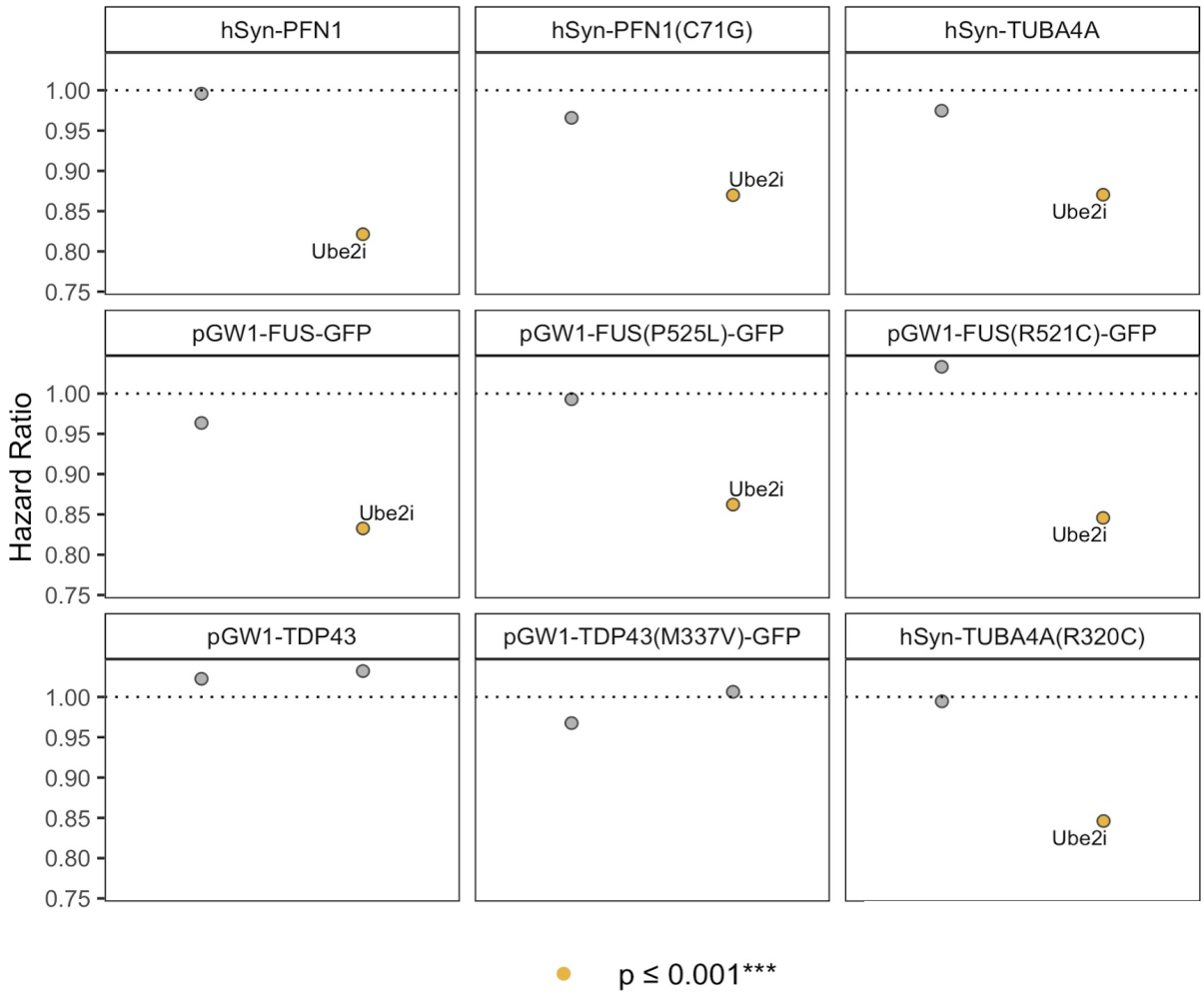

Figure 1. Hazard ratios of neurons expressing different ALS-associated proteins and co-transfected with Ube2i (UBC9) siRNA or non-targeting siRNA. Ube2i siRNA was neuroprotective against wildtype (WT) PFN1, PFN1 C71G, WT TUBA4A, TUBA4A R320C, WT FUS, FUS 525L FUS R521C when compared with non-targeting siRNA (yellow circles vs. grey circles in each graph). However, Ube2i did not reproducibly reduce the hazard ratio of WT TDP43 or TDP43 M337V expressing neurons.

Ube2i knockdown is neuroprotective against WT and mutant TUBA4, WT and mutant FUS, and WT and mutant PFN1, but not WT or mutant TDP43.

In order to evaluate the neuroprotective properties of Ube2i (UBC9) knockdown in patient-derived iPSC-neurons, we rely on our robotic microscopy system and automated image analysis pipeline. Individual neurons are labeled with fluorescent proteins and identified in images, tracked, and cox proportional hazard analysis is used to measure cumulative risk of death. We learned that oftentimes, if iPSC-neurons migrate in the dish, our tracking algorithms may lose the neuron in images and not be able to track them accurately. We have made significant improvement to the way we culture neurons so that migration is minimized. However, this has not been enough for us completely rely on automation in every case. To ensure the accuracy of the tracking, we were manually correcting any changes in our images for most of our experiments. However, this is labor intensive and inefficient. To improve on detecting and quantifying dying neurons and to be able to measure neuroprotective properties of Ube2i (UBC9) siRNA, we developed a different method of quantifying cell death that is as accurate as measuring cumulative risk of death. For this analysis we measure cumulative death rate. We trained a computer neural network (CNN) to identify alive and dead neurons in images from our robotic microscopes. In this method, percent number of alive and dead neurons are measured at each timepoint and quantified over time to produce a cumulative death rate over the course of a multi-day experiment. Cumulative death rates for neurons in one condition are compared with cumulative death rates for neurons in another condition to determine changes in neuronal survival. We compared cumulative hazard and cumulative death rate side by side and found that they produce similar results in our hands. Cumulative death rate analysis can sometimes be more accurate partly because more neurons can be segmented and identified in each image (Figure 2). This is because all neurons are included in the analysis where as for cox proportional hazard analysis, only neurons that are visible in the first time point are counted. This increases sample size and reduces variability.

We find that our new method of analyzing changes in death rate is significantly more efficient because it does not rely on human-curation of images. We are using this method for our experiments moving forward.

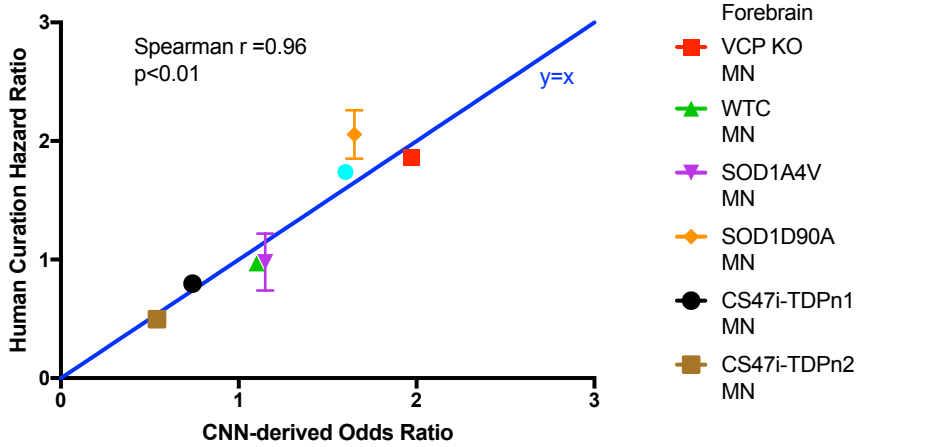

Figure 2. We compared hazard ratios derived from images that were hand-curated by technicians and compared these with odds ratio or derived from our images analyzed by our computer neural network and found that they correlate well. Images used were from iPSC-derived motor neurons (MN) from ALS patients with SOD1, TDP43, and VCP mutations. We included images of iPSC-derived forebrain neurons from Synuclein triplication patient as comparison because forebrain neurons typically migrate less compared with motor neurons.

Aim 2: Validate the role of UBC9 in neurodegeneration in vivo in murine models of ALS.

To test this hypothesis, our initial approach was to inject mouse pups from ALS models with AAV9 expressing a miRNA directed against UBC9. The mice would be monitored to establish the therapeutic effects of UBC9 knockdown on ALS-related phenotypes. The rationale for this aim is that the successful rescue of survival in several ALS mouse models will strongly support UBC9 knockdown as a therapeutic approach for ALS, either through viral RNAi/ASO UBC9 KD or through the identification of small molecules that inhibit UBC9 function.

To assess the effects of UBC9 knockdown, we proposed to utilize two mouse models for ALS based on mutant UBQLN2 and PFN1 based on their ability to recapitulate several human ALS phenotypes. We initially choose to start with the mutant UBQLN2 mouse model based on the fact, through another project, have created an improved mutant PFN1 mouse that is in the final stages of characterization that will subsequently use.

Our initial focus was towards developing, optimizing and characterizing AAV9-miRNA-UBC9 viral vectors. Nine different constructs were made using different miRNA sequences against UBC9 and tested in N2A cells for their level of UBC9 knockdown (Figure 1). Most demonstrated a more than 50% knockdown based on qRT-PCR. Three of these were chosen for injection into mice (mirs 2236, 988, 931) at P0. Unfortunately, all of these resulted in death or sickness of all mice by 3 weeks. Additional attempts resulted in similar results. Analysis of the spinal cord of these mice confirmed lower expression of UBC9 (Figure 2).

As an alternative approach, we decided to cross the UBQLN2 mouse model with UBC9 (a.k.a. UBE2I) hemizygous mice (https://www.jax.org/strain/031573) to obtain decreased levels of expression of UBC9. Full knockout of UBC9 is lethal to mice. These mice are currently being monitored for there therapeutic effect. Due to its ability to detect changes pre-symptomatically, testing by CMAP is being applied every 2-4 weeks. A cross section of the colony at 135 days is shown in Figure 3. However, the mice are currently too young to begin showing any deleterious effect through CMAP.

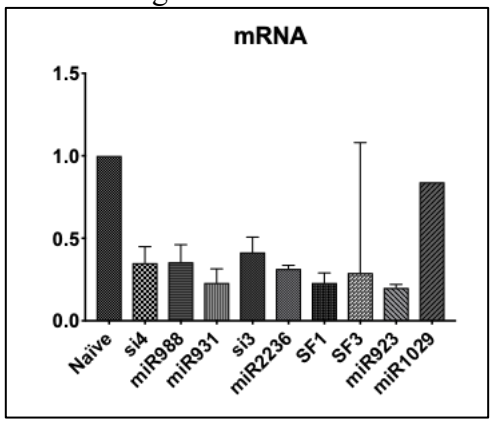

Figure 1

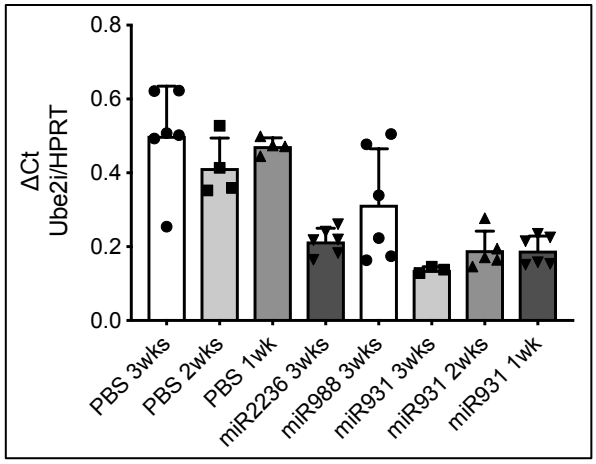

Figure 2

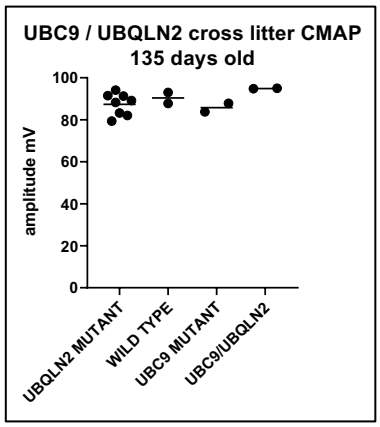

Figure 3

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We plan to evaluate Ube2i (UBC9) knockdown in familial and sporadic ALS patient neurons. We also plan to continue to evaluate the mouse models in vivo.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

For aim 1, we invested some effort in improving our analysis pipeline. For aim 2, we chose to start with the mutant UBQLN2 mouse model because through another project, we have created an improved mutant PFN1 mouse that is in the final stages of characterization that will subsequently use.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We do not anticipate delays.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

We spent less effort on iPSC culturing in this round because we invested time in improving our analysis pipeline for aim1. This reduced our expenditure because while this work required man-power and time, it did not require as much iPSC culturing which is very expensive. We expect that the expenditure will increase in the second year as we will increase iPSC culturing.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals.

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

We developed technique to evaluate neuronal death using computer neural networks and measurement of odds ratio. These methods will be published in the future.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name:	Steve Finkbeiner
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	No Change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

See next page

What other organizations were involved as partners?

Organization Name: UMass Medical School
Location of Organization: Worcester, Massachusetts
Partner's contribution to the project

- *Collaboration* – Subaward with Dr. Landers at UMass. The Landers Lab will directly assess the therapeutic potential of reducing expression, or knocking down (KD) of the UBC9 gene in two mouse models of ALS.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ebrap.org/** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

OTHER SUPPORT

FINKBEINER, STEVEN

PREVIOUS

(Finkbeiner, PI)
Verily Life Sciences (Formerly Google Life Sciences)
Research Agreement: Accelerated Biomarker Development For Parkinson's Disease

09/01/15–Completion of Work
Proprietary

0.9 cal mos

Andrew Conrad
Verily Life Sciences LLC
269 East Grand Ave
South San Francisco, CA 94080

This award ended since last report. Proprietary.

(Finkbeiner, PI)
Vertex Pharmaceuticals Incorporated

05/24/17–04/23/18
Proprietary

0.12 cal mos

Paul Negulescu, Ph.D.
Vertex Pharmaceuticals Incorporated
1010 Torreyana Road
San Diego, CA 92121

This award ended since last report. Proprietary.

(Finkbeiner, PI)
Teva Pharmaceuticals
Characterize the Transcriptomic Signature of Pridopidine in Human Forebrain-like Neurons or Human Neurons with RNAseq

12/16/16–Completion of Work
Proprietary

0.12 cal mos

Irie Grossman, PhD
5 Basel St.
Petach Tikva, Israel, 49131

This award ended since last report.

(Finkbeiner, PI)
Merck Sharp & Dohme Corp.
Research Collaboration between Merck and Gladstone

09/02/15–09/01/18
Proprietary

Effort Reflected in TK Center

Chris Mirescu, PhD
Merck Sharp & Dohme Corp.
33 Avenue Louis Pasteur, Mailstop 8-120
Boston, MA 02115-5727

This award ended since last report.

(Finkbeiner, PI) 11/02/12–11/01/17 (NCX) Effort
ALS Therapy Development Institute Proprietary Reflected in
A Proposal to Screen FDA-approved Drugs in a Primary Neuron TK Center
Model of ALS

Steve Perrin, CEO
ALS TDI
300 Technology Square, Suite 400
Cambridge, MA 02139

This award ended since last report.

R21 NS090395 (Thompson, PI) 07/01/16–06/30/17 0.06 cal mos
UCI/ NIH NCX to 6/30/18
Genome Editing in HD iPS Cells to Reduce Mutant and Total $25,000
Huntington Expression
Role: Consortium PI

Nina Crow, Subcontract Officer
Sponsored Projects Administration
Office of Research
University of California, Irvine
5171 California Avenue, Suite 150
Irvine, CA 92697-7600

This award ended since last report. The major goal of this project is for the Finkbeiner laboratory to perform single cell imaging analysis on HD iPSCs with constitutive and inducible huntingtin knockdown.

R21 NS093236 (Finkbeiner, PI) 09/01/16–07/31/18 0.42 cal mos
NIH/ NINDS $150,000
TRAP1 and FOXO1 as Modifiers of Progranulin and FTLD

Allison Bailey, Grants Management Specialist
NINDS Division of Extramural Research
6001 Executive Blvd., NSC/Rm. 3273
Bethesda, MD 20892-9537

This award ended since last report. The major goals of this project are to determine: 1) whether genetic and pharmacological inhibitors of TRAP1 and FoxO1 stimulate GRN expression in GRN haploinsufficient neurons, and 2) the mechanisms by which TRAP1 and FoxO1 affect GRN expression.

(Finkbeiner, PI) 09/01/17–08/31/18 0.024 cal mos
Hereditary Disease Foundation $75,000
Assessment of WGS-derived Genetic Modifiers in Differentiated HD-derived iPSCs

Travis Carey, Chief Financial Officer
Hereditary Disease Foundation
3960 Broadway
New York, NY 10032

This award ended since last report. The major goal is to identify valid genetic modifiers of Huntington's disease (HD), which may prove easier to target therapeutically than the mutation that causes HD and would provide additional insights into the most important mechanisms of pathogenesis.

R01 NS083390 (Finkbeiner, PI) 09/23/13–12/31/18 (NCX) 0.12 cal mos
NIH/ NINDS $214,732
Automated Longitudinal Single Cell Analysis

Allison Bailey, Grants Management Specialist
NINDS Division of Extramural Research
6001 Executive Blvd. NSC/Rm. 3273
Bethesda, MD 20892-9537

This award ended since last report. The major goals of this project are to: 1) develop a third generation robotic microscope, 2) improve the sensitivity and throughput of the system for high-content screening applications, 3) develop the Bayesian statistical analysis capabilities to further enable understanding of cell-autonomous processes from single cell survival data from high throughput screens, and 4) further develop the technology to study the role of cell non-autonomous processes and single cells in complex tissues.

R01 NS039074 (Finkbeiner, PI) 02/15/13–12/31/17 0.12 cal mos
NIH/ NINDS NCX to 12/31/18
Mechanisms of Huntingtin-induced Neurodegeneration $199,169

Allison Bailey, Grants Management Specialist
NINDS Division of Extramural Research
6001 Executive Blvd., NSC/Rm. 3273
Bethesda, MD 20892-9537

This award ended since last report. The major goals of this project are to: 1) determine how the neuronal protein homeostasis system dynamically responds to mutant huntingtin and assess the effectiveness of the responses, 2) determine how deficits in protein homeostasis lead to neurodegeneration, and 3) adapt powerful single cell longitudinal methods to study protein homeostasis and mutant huntingtin metabolism in brain slice and *in vivo*.

(Finkbeiner, PI) 02/01/18–01/31/19 0.12 cal mos
Alzheimer's Drug Discovery Foundation $150,000
Novel Human FTLD Neuron and Microglia Cell Models for Drug Discovery

Lauren Friedman, PhD
Alzheimer's Drug Discovery Foundation
57 West 57th Street, Suite 904
New York, NY 10019

This award ended since last report. The major goal is to develop neuron and microglia models from human FTLD patient cells with robust phenotypes suitable for high-throughput screening. These new human FTLD cell models can serve as physiologically relevant platforms, creating powerful screening opportunities for drug discovery.

11871.01 (Finkbeiner, PI) 01/30/18–08/31/19 NCX 0.06 cal mos
Michael J. Fox Foundation $179,329
Longitudinal Phenotypic Deficits in iPSC-derived Neurons from PD Patients with Mutations in Parkin and PINK1 Monitored by RM

Todd Sherer, PhD
Michael J. Fox Foundation
Grand Central Station
P.O. Box 4777
New York, NY 10163-4777

This award ended since last report. This major goal of this project is to help to develop a cell-based platform for measuring PINK1- and Parkin-related mitophagy and neurodegeneration phenotypes that could be used to discover and develop PINK1 and Parkin activators.

CURRENT

Target ALS/ Denali (Finkbeiner, PI) 01/01/17–12/31/19 0.06 cal mos
Targeting Stress Granule Dynamics for Familial and Sporadic ALS $150,000

Manish Raisinghani, President
Target ALS Foundation, Inc.
Radio City Station
PO Box 1598
New York, NY 10101-1598

This award received an additional year of funding since last report. The major goal of this project is to identify compounds that can restore normal stress-granule dynamics in the presence of pathogenic insults like mutant TDP43 and progress these towards clinical studies.

(Finkbeiner, PI) 01/01/17–12/31/19 0.06 cal mos
Target ALS Foundation $100,000
Exploiting Yeast to Discover Small-molecule Drugs for ALS Caused by Aberrant TDP-43, FUS, and c9orf72 Dipeptide Repeat Protein Homeostasis

Manish Raisinghani, President
Target ALS Foundation, Inc.
PO Box 1598
Radio City Station
New York, NY 10101-1598

This award received an additional year of funding since last report. The major goal is to optimize drug-like small molecules that rescue toxicity by aberrant TDP-43,FUS or (PR) proteostatis through more sophisticated assays to identify in vivo tool compounds.

W81XWH-15-1-0158 (Finkbeiner, PI) 09/15/15–09/14/18 0.12 cal mos
Department of Defense $327,837
Development of Novel Neuronal Autophagy Inducers to Block Neurodegeneration and Treat ALS NCX to 11/30/19

Brett Chaney, Science Officer, ALSRP
General Dynamics Information Technology (GDIT)
Congressionally Directed Medical Research Programs (CDMRP)
1077 Patchel Street
Fort Detrick, MD 21702

This award received a no cost extension since last report. The major goals of this project are to: 1) identify the NAIs that most effectively stimulate autophagy in human i-neurons and protect rodent and human ALS i-neurons and ALS i-astrocytes from degeneration, 2) select NAIs that most effectively stimulate autophagy in CNS *in vivo*, and 3) test NAIs *in vivo* for efficacy in reducing motor deficits, neuropathology and increasing survival of SOD1 (G93A) and TDP43 (A315T) transgenic mice.

(Finkbeiner, PI) 10/01/16–12/31/19 NCX 0.06 cal mos
ALS Finding A Cure $284,793
Answer ALS: iPSC Phenotyping and Whole Genome Sequencing

Christine Collins
23 Barry Place
Stamford, CT 06902

This award received a no cost extension since last report. The main goal of this project is for the Finkbeiner laboratory to assist with two major arms of the study: iPSC phenotyping and Whole Genome Analysis.

(Finkbeiner, PI/Director) 12/11/08–07/31/21 0.24 cal mos
Koret Foundation $600,000
Taube/ Koret Center for Neurodegenerative Disease Research

This award received additional funding since last report. The major goal of this project is to establish a Center for HD research with the specific goal of advancing therapeutic leads from our basic science research programs to a stage where partnership with industry is warranted.

Torey Industries (Finkbeiner, PI) Sponsored Research Agreement Proprietary	04/01/19–09/30/21 Proprietary	Effort reflected in TK Center
Takeda Pharmaceutical (Finkbeiner, PI) Sponsored Research Agreement Proprietary	12/16/18–12/15/20 Proprietary	Effort reflected in TK Center
12738 (Finkbeiner, PI) Michael J. Fox Foundation PD Head Start Program: Combining Functional Genomics with Human Neuron Models to Prioritize PD Risk Variants	06/01/16–05/31/18 NCX to 4/30/20 $912,604	0.06 cal mos

Todd Sherer, PhD
Grand Central Station
P.O. Box 4777
New York, NY 10163-4777

This award received a no cost extension since last report. The major goals of this project are to: 1) perform functional genomics by leveraging existing genomic, transcriptomic and proteomic data from Parkinson's disease (PD) patients, and 2) predict novel genetic modifiers that will be validated in cellular models that recapitulate the diseas.1e process. During this project we aim to establish a scalable infrastructure that could be built into a larger effort by partnering with NINDS and industry groups.

15-LGCA-231 (Finkbeiner, PI) Amyotrophic Lateral Sclerosis Association (ALSA) Identification and Validation of Therapeutic Targets and Development of Therapeutics for ALS	02/01/17–01/31/19 $909,091 NCX to 05/31/20	0.12 cal mos

Lucie Bruijn, PhD, Chief Scientist
The ALS Association
27001 Agoura Road, Suite 250
Calabasas Hills, CA 91301

This award received a no cost extension since last report. The major goals of this project are to: 1) advance the therapeutics program closest to a Phase I clinical trial until it fails or is partnered for clinical development, 2) prioritize and advance a portfolio of back up therapeutics programs for ALS to replace the lead program when it is partnered, and 3) expand and develop the preclinical iPSC-based drug discovery platform for ALS.

U54 NS091046 (Thompson, PI) 09/30/14–06/30/20 0.48 cal mos
UCI/ NIH Data Generation: $105,000
Neuron and Glial Cellular Signatures from Normal and Diseased iPS Cells Data Analysis: $16,831
Role: Co-investigator

Nina Crow, Subcontract Officer
Sponsored Projects Administration
Office of Research
University of California, Irvine
5171 California Avenue, Suite 150
Irvine, CA 92697-7600

No change. With the establishment of a NeuroLINCS Consortium the major goals of this project are to: 1) generate data for cell signatures from human iPSC derived neurons, astrocytes and oligodendrocytes from healthy and diseased patients at baseline and in response to perturbagens with transcriptomics, epigenomics, whole genome sequencing, proteomics and cell-based assays, including high-content longitudinal single cell analysis, 2) build cell signatures that convey the key features that distinguish the state of a cell and determine its behavior, and 3) integrate the resources and results generated at the LINCS site with the broader LINCS consortium.

R37 NS101996-01 (Finkbeiner, PI) 04/01/17–03/31/21 0.90 cal mos
NIH $325,993
R37 Javits Award
Rare Variant Whole Genome Analysis and iPSC Validation of Putative Genetic Modifiers of Huntington Disease

James Washington, Grants Management Officer
NINDS Division of Extramural Research
6001 Executive Blvd. NSC
Bethesda, MD 20892-9537

No change. The major goal of this project is to identify novel genetic modifiers that either enhance the toxicity of mutant huntingtin, the cause for HD, or slow the neurodegeneration induced by mutant huntingtin.

A-10612 (Finkbeiner, PI) 03/01/17–02/29/20 0.12 cal mos
CHDI $722,432
Research Agreement

Ruth Basu
CHDI Foundation, Inc.
350 Seventh Avenue, Suite 200
New York, NY 10001

This award received an additional year of funding since last report. The major goal of this project is to analysis of HD phenotypes in stem cell models using quantitative microscopy.

RF1 AG058476-01 (Finkbeiner, PI) 09/15/17–03/31/22 0.90 cal mos
NIH/ NINDS $335,000
Dysfunction of the Autophagy-lysosomal Pathway as a Common Mechanism of Neurodegeneration

Richard Proper, Grants Management Specialist
NINDS Division of Extramural Research
6001 Executive Blvd.
Bethesda, MD 20892-9537

No change. The major goal of this project will be to understand better how neurons that are most vulnerable in common neurological diseases, become ineffective at removing disease causing misfolded proteins, leading to impaired neuron function and ultimately cell death.

RF1 AG056151-01 (Duff, Finkbeiner, MPI) 09/30/2017–06/30/22 0.90 cal mos
Columbia University/NIH/NIA $194,877
Tauopathy in AD and FTD – Molecular Determinants of Phenotypic Diversity

Rudina Odeh-Ramadan, Associate Vice President for Research Administration
154 Haven Ave, Third Floor
New York, NY 10032-3702

Newly awarded since last report. The major goal of this project is for Dr. Finkbeiner to be the partnering PI on the grant with Dr. Karen Duff. Overall, the R01 is focused on elucidating mechanisms of tau-dependent propagation and neurodegeneration.

(Finkbeiner, PI) 11/16/17–11/16/20 0.12 cal mos
Eli Lilly Proprietary

Daniel Skovronsky
10300 Campus Point Drive
San Diego, CA 92121

FA870215D0001 (Thorsen, PI) 04/01/18–03/31/19 0.12 cal mos
MIT Lincoln Laboratory/ Department of the Air Force $67,135
Artificial Gut NCX to 07/31/20
Role: Co-investigator

Sara Eagan
244 Wood Street
Lexington, MA 02420-9180

Newly awarded since last report. The major goal of this project is for the Dr. Finkbeiner's laboratory to work with Dr. Thorsen's lab to investigate the relationship between the gut and the nervous system. Using Dr. Thorsen's artificial gut platform, this project aims to study the link between the metabolome and Parkinson's disease with the aid of automated microscopy.

RF1 AG058447-01 (Finkbeiner, PI) 06/01/18–05/31/21 0.90 cal mos
NIH/ NIA $383,611
Discovery of Novel Drugs that Increase Tau Clearance to Treat Alzheimer's Disease

Jessica Perez, Grants Management Specialist
National Institute on Aging
Building 31, Room 5C27
31 Center Drive, MSC 2292
Bethesda, MD 20892

Newly awarded since last report. The major goal of this project is to develop innovative *in vivo* assays to discover small-molecule drugs to treat neurodegenerative diseases (e.g., Alzheimer's disease and Frontotemporal dementia, the two most prevalent dementia-related diseases).

W81XWH-18-1-0696 (Finkbeiner, PI) 09/15/18–09/14/20 0.24 cal mos
Department of Defense ALSRP $500,000
Validating UBC9 as a Molecular Target to Develop a Therapy for All Forms of ALS

Elfreda Nymn, Grants Specialist
US Army Medical Research Acquisition Activity
820 Chandler Street
Fort Detrick, MD 21702-5014

This award. The major goal of this project will be to develop novel disease-modifying small-molecule drugs that block neurodegeneration in ALS to slow disease progression.

1761941 (Finkbeiner, PI) 08/01/18–07/31/23 0.36 cal mos
National Science Foundation $154,813
Combining Heterogeneous Data Sources to Identify Genetic Modifiers of Diseases

Ilonka Karasz, Grant Official
Division of Grants and Agreements
2415 Eisenhower Avenue
Alexandria, VA 22314

Newly awarded since last report. The major goal of this project will develop tools to combine genome association studies with other sources of data, such as family-based genetic studies that identify rare variants and transcriptomic or proteomic studies that capture gene expression signatures in disease, to find genetic modifiers that would be entirely missed by the use of genetics alone.

U01MH115747 (Krogan/Ideker/Kampmann/Kriegstein/Willsey) 09/05/18–06/30/23 0.90 cal mos
Psychiatric Cell Map Initiative: Connecting Genomics, $150,000
Subcellular Networks and Higher Order Phenotypes
Role: Co-Investigator

Sally Brown, Contracts & Grants Officer
600 16th Street,
San Francisco, CA 94158

Newly awarded since last report. The major goal of this project will be to develop this integrative platform by initially focusing on autism spectrum disorder, as recent discoveries offer unprecedented opportunities to develop a deeper understanding of the pathway-level biology. The characterization of high-resolution physical and genetic interaction networks will build on these findings to advance autism spectrum disorder neurobiology. Understanding the key molecular networks underlying different neuropsychiatric disorders will be critical to disentangling their pathobiology and identifying biomarkers and therapeutics.

(Finkbeiner, PI) 01/01/19–2/29/20 0.12 cal mos
Michael J. Fox Foundation $196,539
Applications of Deep Learning to Assess PD Pathology

Todd Sherer, PhD
Grand Central Station
P.O. Box 4777
New York, NY 10163-4777

Newly awarded since last report. We propose to train deep learning convolutional neural networks on pathology samples from Parkinson's disease (PD) patients in an effort to develop an algorithm that could improve the diagnosis and staging of PD more robustly and reliably than is currently possible. If successful, the algorithm could be disseminated and scaled, potentially providing an earlier diagnosis and a more quantitative, uniform and inexpensive tool for assessment of samples in large clinical studies.

W81XWH-19-1-0095 (Finkbeiner, PI) 03/15/19–09/30/20 0.6 cal mos
DOD USAMRAA $131,992
The role of epigenetics and methylation near the DMPK gene in causing neurodegeneration in Myotonic dystrophy type 1

Office of the Congressionally Directed Medical Research Programs (CDMRP)
1077 Patchel Street (Building 1077)
Fort Detrick, MD 21702-5024

Newly awarded since last report. We propose to identify mechanisms by which mutations in the dystrophia myotonica protein kinase gene (DMPK) cause Myotonic dystrophy type 1 (DM1), a FY18 PRMRP Topic Area. DM1 is the most common form of adult onset muscular dystrophy and is caused by an expanded "CTG" repeat in the DMPK. A number of studies have suggested that impaired RNA processing contributes to disease pathology in DM1.

1 RF1 AG060765-01A1 (Finkbeiner, PI) 04/15/19–2/29/24 0.9 cal mos
Novel Therapeutic Approaches to Increase Progranulin Levels in Brain to Treat FTLD and AD $353,044

Richard Proper, Grants Management Specialist
National Institute on Aging
Building 31, Room 5C27
31 Center Drive, MSC 2292
Bethesda, MD 20892

Newly awarded since last report. The goal of our proposed study is to test the hypothesis that progranulin (PGRN) and small molecule drugs that increase PGRN expression in neurons will be neuroprotective and block neurodegeneration in FTD and possibly AD. We propose to test small molecule drugs for effcalcicacy in increasing PGRN levels in brain to determine if they slow neurodegeneration both in vitro in murine and human cell models of FTD as well as in vivo in mouse models of FTD.

P01 AG054407-01 (Morimoto, PI)
Northwestern University / NIH / NIA
Proteostasis in Aging and Neurodegenerative Disease
Role: Consortium PI

Georgette Pliml, Project Coordinator
Department of Molecular Biosciences, Northwestern University
2205 Tech Drive, Hogan 2-100
Evanston, IL 60208-3500

Newly awarded since last report. The major goals of our Aim and Core for this project is to 1) determine how progranulin deficiency affects the autophagosomal/lysosomal pathway and other proteostasis network components, 2) define the role of progranulin in the proteostasis of neurodegenerative disease-causing proteins, and 3) discover, validate and characterize new small molecules that modulate the autophagosomal/lysosomal pathway.

1 R01 AG067025 (Finkbeiner, PI) 09/15/19–06/30/23 1.2 cal mos
Understanding the molecular mechanisms that contribute to neuropsychiatric symptoms in Alzheimer's Disease $182,337

Icahn School of Medicine at Mount Sinai
1470 Madison Avenue, Box 1639
New York, NY 10029
(212) 241-6696

Newly awarded since last report. Neuropsychiatric symptoms (NPS) are core features of Alzheimer's disease (AD) and related dementias, that contributes to early institutionalization and causes substantial caregiving and caregiver burden. NPS are among the earliest symptoms of AD and other dementias, and there are indications that presence of NPS during mild cognitive impairment (MCI) might increase the risk of dementia. Despite decades of research, reliable treatments for NPS in the context of AD and other dementias have not been found. Therefore, a better understanding of the molecular mechanisms and pathways underlying NPS in the context of AD and other neuropsychiatric illnesses is a critical next step to identify reliable biomarkers that could lead to novel therapeutics.