

AWARD NUMBER: W81XWH-16-1-0550

TITLE: Treating ALS by targeting pathological TDP-43

PRINCIPAL INVESTIGATOR: Brian C. Kraemer, PhD

RECIPIENT: Seattle Institute for Biomedical and Clinical Research
Seattle, WA 98108

REPORT DATE: September 2017

TYPE OF REPORT: Annual

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

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE September 2017		2. REPORT TYPE Annual		3. DATES COVERED 1 Sep 2016 - 31 Aug 2017	
4. TITLE AND SUBTITLE Treating ALS by targeting pathological TDP-43				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-16-1-0550	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Brian C. Kraemer, PhD VA Puget Sound Health Care System / University of Washington E-Mail: kraemerb@uw.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Seattle Institute for Biomedical and Clinical Research 1660 S. Columbian Way, S-151F Seattle WA 98108-1532				8. PERFORMING ORGANIZATION REPORT NUMBER KB28A-PRSCI-1	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT TAR DNA-binding protein 43 kD (TDP-43) is the major aggregating disease protein in amyotrophic lateral sclerosis (ALS). Our previous work demonstrated pS409/410 TDP-43 mediates motor neuron toxicity of familial ALS-causing TDP-43 mutations, and identified two well conserved kinases, tau tubulin kinase 1 and tau tubulin kinase 2 (TTBK1/2). Kinases regulating TDP-43 phosphorylation present an attractive target for therapeutic intervention in ALS. Development of brain penetrant TTBK1 and TTBK2 inhibitors may provide a viable strategy for intervening in ALS. We have completed the primary screen of Specific Aim 1: Identification of TTBK1/2 selective kinase inhibitors . A collection of investigational kinase inhibitor drugs and CNS penetrant drugs (~56,000 compounds in total) was screened to identify compounds quantitatively decreasing TTBK1 activity <i>in vitro</i> . Dose-validation analysis of hits is ongoing at Quellos High Throughput screening core. Subsequent follow-up analysis in simple model systems will be completed in the coming months.					
15. SUBJECT TERMS TAR DNA-binding protein 43 kD, TDP-43, tau tubulin kinase 1, TTBK1, tau tubulin kinase 2 TTBK2, amyotrophic lateral sclerosis, ALS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	12	19b. TELEPHONE NUMBER (include area code)

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

Preclinical development of therapeutics targeting pathological TDP-43. Pathological TDP-43 in either cortical or motor neurons causes neurodegenerative changes in a group of disorders known as TDP-43 proteinopathies which include frontotemporal lobar degeneration and amyotrophic lateral sclerosis (ALS). The progressive dementia and/or motor dysfunction caused by TDP-43 proteinopathy disorders have no effective treatment, cause severe disability, and lead to premature death. *To date no clinical trials for ALS have specifically targeted pathological TDP-43 which is the primary neuropathology evident in over 90% of sporadic ALS cases and most familial ALS cases.* To fill this gap, we propose to develop specific kinase inhibitor targeted neuroprotective strategies aimed at ameliorating pathological TDP-43 in ALS.

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

TDP-43, TTBK1, TTBK2, ALS

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.

Specific Aim 1: Identification of TTBK1/2 selective kinase inhibitors. A large collection of investigational kinase inhibitor drugs and CNS penetrant drugs (~56,000 compounds in total) will be screened to identify compounds quantitatively decreasing TTBK1/2 activity *in vitro*.

Specific Aim 2: Evaluation and optimization of hit compounds. We will explore whether TTBK1/2 small molecule inhibitors identified in Aim 1 can reverse TDP-43 pathology in simple cellular models of TDP-43 aggregation and hyper-phosphorylation. Compounds will be subject to medicinal chemistry optimization. Compounds effective against TDP-43 pathology in simple systems will be tested for brain penetrance in mice.

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.

We have completed the screening portion of Aim 1 and are initiating the validation portion of Aim 2. The status of each Major Task is summarized as follows:

1.1 Specific Aim 1/Major Task 1: High throughput screen for TTBK1/2 inhibitors. We have completed the primary screening of KINASet Library.

1.2 Specific Aim 1/Major Task 2: Hit compound selectivity assay. In vitro response analysis of compounds to determine IC50. Milestone: TTB1/2 selective kinase inhibitors identified. This task was delayed during some equipment repair in the Quellos High Throughput Screening Core. The equipment is now repaired. Estimated time to completion is 1 month.

2.1 Specific Aim 2/Major Task 1: Evaluation of TTBK1/2 inhibitor target engagement in cellular models. The dose response analysis of inhibitor effects on pTDP accumulation in human cells was delayed because of equipment problem at the Quellos High Throughput Screening Core. These issues are now resolved and we expect progress to resume.

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

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state “Nothing to Report.”

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

Complete Aim 2 and Task 1.2 as planned (dose response analysis of inhibitor effects on pTDP accumulation in human cells). This will complete the SOW.

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 at this time.

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 yet. IP considerations pending completion of the screen.

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.

Nothing to report. There were no changes in approach during the reporting period.

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 experienced delays in initiation of screening at Quellos High Throughput Screening core, due to equipment failure at that facility. The issue has been resolved and screening is now ongoing.

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.

Delays in screening at the Quellos High Throughput Screening core (due to equipment failure at that facility) have resulted in request for no cost extension. The expenditures associated with the Quellos High Throughput Screening core’s services have been delayed into the no cost extension year, as has some of the planned Research Scientist effort. We anticipate using the remaining funds in the no cost extension period to support the approved aims of the project.

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

Not applicable

Significant changes in use or care of vertebrate animals.

Not applicable

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 yet. Expect to pursue publication of results after Task 1.2 and Aim 2 are completed over the coming year.

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 yet. Expect to pursue publication of results after Task 1.2 and Aim 2 are completed over the coming year.

- **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.

Nothing to report

- **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.”

Example:

Name: Mary Smith
 Project Role: Graduate Student
 Researcher Identifier (e.g. ORCID ID): 1234567
 Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.
 Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award).

Name: Brian C. Kraemer, PhD
 Project Role: PI
 Researcher Identifier (e.g. ORCID ID): 0000-0002-2252-7634
 Nearest person month worked: 1
 Contribution to Project: Dr. Kraemer is the PI. He has direct overall responsibility for performance of all aspects of the project. He also analyzes data, directs Quellos’ screening, and oversees staff effort and every other aspect of the project.
 Funding Support: VA salary

Name: Timothy Strovas, PhD
 Project Role: Research Scientist
 Researcher Identifier (e.g. ORCID ID): None
 Nearest person month worked: 3
 Contribution to Project: Dr. Strovas validated hit compounds in cellular systems. Furthermore, he has and will continue to make significant intellectual contributions to the project, including co-authoring manuscripts.
 Funding Support: N/A – supported by this award.

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

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Dr. Kraemer’s effort on A2014438S from the Bright Focus Foundation (Dopamine signaling controls pathological tau) reduced from 20% to 5% when that project went into no-cost extension. The new end date is 6/30/2018.

Dr. Kraemer’s effort on A2014438S from the National Institute of Neurological Disorders and Stroke (Unfolded protein response activation protects neurons against pathological tau) reduced from 20% to 10% when that project went into no-cost extension. The new end date is 6/30/2018.

A new project began:

RF1AG055474 (Kraemer)

4/1/2017 – 3/31/2022

25% Effort

National Institute on Aging

MSUT2 modulates pathological tau in AD and model organisms

The Specific Aims of this project are to: 1) Characterize the consequences of MSUT2 knockout in mouse models of tauopathy. 2) Determine the effect of increased MSUT2 activity on tau neuropathology and behavioral phenotypes in mice. 3) Dissect the molecular mechanisms of MSUT2 modulation of tauopathy. Completion of the project as proposed will demonstrate the importance of MSUT2 in tauopathy. We will also gain significant understanding of the molecular mechanisms involved in MSUT2 modulation of tau pathology in diverse organisms ranging from *C. elegans* to humans.

Grant Point of Contact:

Grants Management Specialist: Kathleen Moy

Email: moyk@mail.nih.gov Phone: 301.827.2856

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner's contribution to the project (identify one or more)

- Financial support;
- In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);
- Facilities (e.g., project staff use the partner's facilities for project activities);
- Collaboration (e.g., partner's staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and
- Other.

Nothing to report

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://ers.amedd.army.mil> 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.

Nothing to report

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.

Nothing to report