AWARD NUMBER: W81XWH-18-1-0684

TITLE: Restoring Neuromuscular Junction Integrity to Alleviate ALS Progression

PRINCIPAL INVESTIGATOR: Jingsong Zhou

CONTRACTING ORGANIZATION: University of Texas at Arlington

REPORT DATE: Oct 2019

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2019	Annual	09/30/2018 - 09/29/2019

4. TITLE AND SUBTITLE

Restoring Neuromuscular Junction Integrity to Alleviate ALS Progression

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0684

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Jingsong Zhou

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University of Texas at Arlington
701 S. Nedderman Drive, Box 19145
Arlington, TX 76019-0145

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

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

ALS is a fatal neuromuscular disease. Failure of the respiratory muscle is a main cause of mortality in ALS patients. Defects in neuromuscular junctions (NMJs) and progressive NMJ loss occur at early stages, thus stabilizing and preserving NMJs represents a potential therapeutic strategy to slow ALS disease progression. This project is aim to explore a potential role of a muscle-derived protein MG53 in maintaining the NMJ and diaphragm muscle membrane integrity, and to establish the efficacy and safety for using recombinant human MG53 protein (rhMG53) to treat ALS in the mouse model. Since MG53 is already present in human blood circulation under normal physiologic conditions, therapeutic approach with modulation of MG53 function or systemic administration of rhMG53 can potentially be a safe biologic reagent for treatment of ALS.

15. SUBJECT TERMS

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
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

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TABLE OF CONTENTS

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

ALS is a fatal neuromuscular disease. Failure of the respiratory muscle is a main cause of mortality in ALS patients. Defects in neuromuscular junctions (NMJs) and progressive NMJ loss occur at early stages, thus stabilizing and preserving NMJs represents a potential therapeutic strategy to slow ALS disease progression. While mitochondria-mediated reactive oxidative species (ROS) links to the etiology of ALS, the mechanisms that underlie NMJ degeneration in ALS are largely unknown. During respiration, the diaphragm constantly undergoes contraction-relaxation, a process that leads to injury to the muscle membrane. Inadequate repair of injury to the sarcolemma can disrupt NMJ integrity and contribute to diaphragm wasting in ALS. MG53 is an endogenous protein in human body that serves essential roles in nucleating assembly of repair patches at membrane injury sites. Genetic ablation of MG53 results in defective membrane repair and tissue regenerative capacity. A series of studies have shown that recombinant human MG53 (rhMG53) protein protects various cell types against membrane disruption when applied to the extracellular environment in animal models. We ***hypothesize that MG53-mediated membrane repair contributes to maintenance of NMJ integrity in ALS***. Since NMJ is an active site of neuron/muscle crosstalk, we postulate that membrane repair defects originate from NMJ. A vicious cycle of mitochondrial dysfunction/membrane repair defects leads to increased vulnerability of NMJ to stress-induced injury as part of ALS pathology. Thus, this project has two specific aims: (1) To elucidate the physiological role of MG53-mediated membrane repair in ALS. Specifically, the SOD1G93A mice will be cross-bred with MG53 knockout and ctPA-MG53 mice to evaluate MG53's physiological role in regulating the degeneration of NMJ associated with ALS progression. These studies will test whether elevated level of MG53 in circulation has protective role for NMJ integrity in ALS. (2) To conduct proof-of-concept study testing rhMG53 as a novel therapeutic means for improving NMJ integrity to treat ALS. We propose to establish the efficacy and safety for using rhMG53 protein to treat ALS in the mouse model. Since MG53 is already present in blood circulation under normal physiologic conditions, therapeutic approach with modulation of MG53 function or systemic administration of rhMG53 can potentially be a safe biologic reagent for treatment of ALS.

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

ALS (Amyotrophic Lateral Sclerosis), NMJ (neuromuscular junction), MG53, rhMG53 (recombinant human MG53), Mitochondria, Skeletal muscle

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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.*
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.

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

Major activities, specific objectives, significant results and the future plan

Aim 1: Elucidate the physiological role of MG53-mediated membrane repair in ALS.

Subtask 1.1: Cross-breeding of *SOD1*^{G93A} mice with *mg53-/-* and ctPA-MG53 mice (1-18 months)
Subtask 1.2: Dissect the role of MG53 in preservation of muscle and NMJ integrity in ALS (6-18 months)
Subtask 1.3: Dissect the function of MG53 in maintenance of mitochondria function in ALS (12-24 months)

Milestones:
1) Physiologic role of MG53 in ALS established. (On going)
2) ALS mice with knockout of MG53 show exacerbated diaphragm muscle injuries. (On going)
3) Elevated MG53 in circulation preserves NMJ integrity in ALS mice. (On going)

1. Establishing the *mg53-/-* and ctPA-MG53 in the University of Texas at Arlington (UTA): Dr. Zhou's lab was relocated from Kansas City University (KCU) to UTA in July 2018. The DOD grant was relinquished from KCU to UTA. The fund started on March 2019. Following the MTA (Material Transfer Agreement between the Ohio State University (OSU) and UTA) signed on Feb 11, 2019, the *mg53-/-* and ctPA-MG53 mice were transferred to UTA on March 2019. After the quarantine and foster breading, the mice are in the process of back-crossing to the same gene background (B6SJL) of the ALS SOD1^{G93A} mice used in Zhou lab. We now completed 1st round of back-crossing.

2. Evaluating the physiological role of MG53 in preserving NMJ in ALS: Our next step is to cross G93A with *mg53-/-* and ctPA-MG53 to generate G93A/*mg53-/-* and G93A-ctPA-MG53. After we obtained the double transgenic mice, we will conduct physiological study to evaluate whether missing or elevated MG53 in circulation affect the muscle function, NMJ integrity and the life span of ALS G93A mice.

Aim 2: Conduct proof-of-concept study testing efficacy and safety of rhMG53 to treat ALS in mice.

Subtask 2.1: Production and quality controls of rhMG53 and PEG-rhMG53. (1-12 months)
Subtask 2.2: Pharmacokinetic (PK) assessment of PEG-rhMG53 in ALS mice. (6-12 months)
Subtask 2.3: In vivo efficacy and safety assays with rhMG53 and PEG-rhMG53 in ALS mice. (6-24 months)

Milestones:
1) Produce 2 grams of rhMG53, sufficient for pre-clinical studies (Completed)
2) In vitro QC assays to ensure purity and function of rhMG53. Endotoxin level of rhMG53 < 10 EU/mg (Completed)
3) PEGylation improves PK of rhMG53 in ALS mice (Completed)
4) Intravenous or subcutaneous administration of rhMG53 or PEG-rhMG53 improves integrity of diaphragm and NMJ in ALS mice. (On going)
5) Repetitive intravenous or subcutaneous administration of rhMG53 does not produce adverse effects in ALS mice. (On going).

1. We have produced 2 grams of the rhMG53 protein, which is sufficient for the preclinical studies.

2. We conducted quality control assays with SDS-PAGE and RP-HPLC to ensure that the rhMG53 protein is >98% purity. In vitro membrane damage assay was used to determine the function of the rhMG53 in repair of cell membrane injuries; the efficacy is comparable to our published studies in Science

Translational Medicine (Weisleder N, et. al., 2012; Duann P, et. Al., 2015). The endotoxin level of the rhMG53 protein is ~2-5 EU/mg.

3. We performed PK studies with the PEGylated rhMG53 in rats, and found that pegylation improved the serum half-life of rhMG53 from 0.5 hour to 12 hours. Data were included in a manuscript under consideration for publication in ***Nature Medicine***.

4. Intravenous administration (IV) of PEG-rhMG53 in ALS G93A mice improved the integrity of diaphragm and NMJ, and extended the life span of ALS muscle. As shown in **Fig 1a.** G93A littermate mice at the age of disease onset (3-month old) were divided into two groups, one receiving PEG-rhMG53 (2 mg/kg, every other day for 30 days), and the other receiving saline as a control. One-month treatment of PEG-rhMG53 significantly extended the life span of G93A mice from 124±2 days (saline) to 137±2 days (PEG-rhMG53). These Data were included in a manuscript under consideration for publication in ***Nature Medicine***.

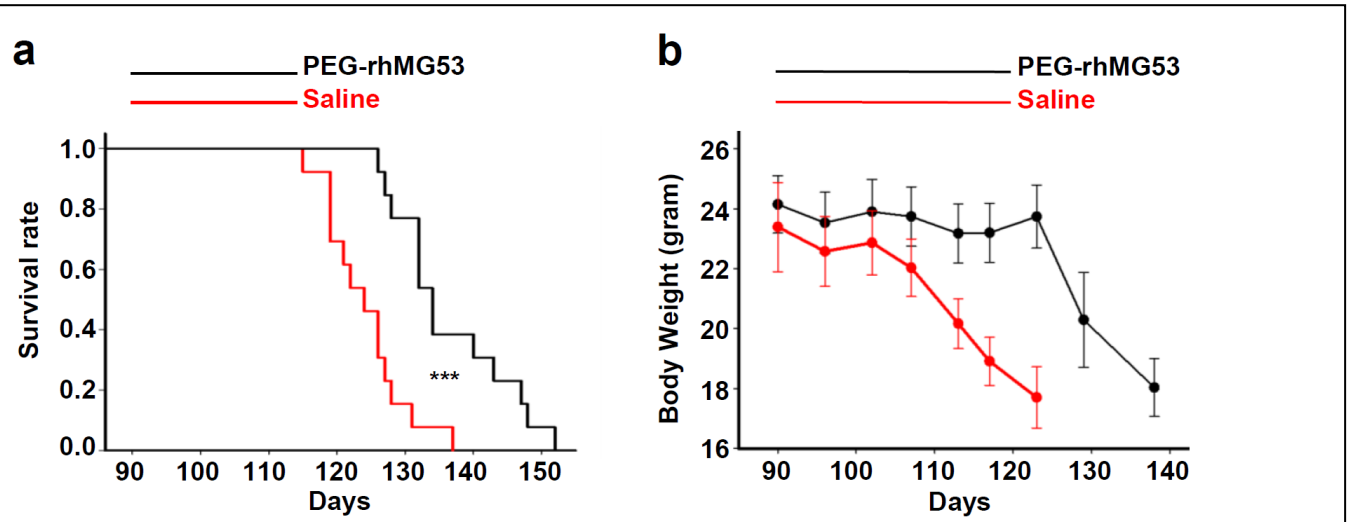

Fig 1. (**a**) Survival curve of G93A mice with one-month PEG-rhMG53 or saline treatment. (n=13 pairs of G93A littermates, ***P<0.001). (**b**). Bodyweight changes of G93A mice receiving PEG-rhMG53 or saline treatment. ***P<0.001

5. Repetitive intravenous or subcutaneous administration of PEG-rhMG53 does not show adverse effects in ALS mice. As shown in **Fig. 1b**., during the one-month PEG-rhMG53 treatment, the G93A mice maintained their body weight, whereas mice receiving saline control showed progressive decline of body weight. In the coming year, we plan to conduct pathological studies in multiple organs with repetitive intravenous administration of rhMG53 in ALS mice to assess the safety profile of rhMG53 in the disease model.

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.

Professional Development Activity: The senior research scientist, Jianxun Yi (also the first author of the manuscript submitted to *Nature Medicine*) attended the **10th World Congress on Targeting Mitochondria 2019**.

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

4. **IMPACT:** D*escribe 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 PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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.

No significant changes

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.

No delays at this time point

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.

No significant changes

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

No changes

Significant changes in use or care of vertebrate animals

No changes

Significant changes in use of biohazards and/or select agents

No changes

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).*

  One manuscript submitted to ***Nature Medicine***
  Title: "MG53 preserves neuromuscular junction integrity to alleviate ALS disease progression"
  Authors: Jianxun Yi, Ang Li, Xuejun Li, Ki-Ho Park, Xinyu Zhou, Frank Yi, Yajuan Xiao, Dosuk Yoon, Tao Tan, Lyle W. Ostrow, Jianjie Ma and Jingsong Zhou

  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).*

  None

  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.*

  Present one abstract at an international meeting "10th World Congress on Targeting Mitochondria 2019".
  Title: "Mitochondria-mediated multi-organ crosstalk in ALS"
  Authors: Jianxun Yi, Ang Li, Xuejun Li, and Jingsong Zhou

- **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.

None

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

None

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. 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.

None

- **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;*
  - *physical 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.*

None

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.)*

Jianjie Ma, Co-I, from the Ohio State University.
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?
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.

No changes

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 Principal Investigator (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.*

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.