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TITLE: Generation of a Mouse Model to Investigate IL-6 Trans-Signaling in ALS

PRINCIPAL INVESTIGATOR: Gregory Hawkins

**CONTRACTING ORGANIZATION: Wake Forest University Health Sciences
Winston-Salem, NC 27157**

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14. ABSTRACT 1) IL6 transsignaling plays a potential protective role for motoneurons in the periphery, while later when extracellular levels of IL6 increase with increased muscle atrophy and decreased lung function, transsignaling promotes a breakdown in the blood brain barrier that fosters IL6 transsignaling in the CNS that can promote disease progressions through glial activation. 2) Individuals with increased levels of soluble receptor such as those with enhanced shedding due to IL6R polymorphism will be more susceptible to IL6 transsignaling and will have faster disease progression. 3) Blocking the effects of IL6 transsignaling will reduce disease progression rates and disease severity.					
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1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

We hypothesize that IL6 transsignaling plays a role in the progression of ALS by affecting the rate of NMJ denervation, glial cell activation, and MN degeneration. Given the difficulty studying humans with ALS and collecting samples critical to studying active disease, we are proposing to utilize the SOD1^{G93A} mouse model of ALS to study the effects of IL6 transsignaling on disease severity and progression. Successful execution of this study will define the role of IL6 as an effector of ALS severity and progression, and will provide new information on how to target and treatment ALS using therapeutics that target and block the detrimental effects of IL6 transsignaling.

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

Amyotrophic lateral sclerosis, ALS, CRISPR mouse model, IL6 trans-signaling, SOD1

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.

Goals:

1. Perform a systemic examination of IL6 transsignaling in both initiation and progression of ALS
2. Create an ALS mouse model where IL6 transsignaling is increased, thus modeling those individuals that have inherited the IL6R polymorphism, and determine if disease pathology is altered.
3. Treat the ALS/transsignaling mouse model with the transsignaling inhibitor, soluble gp130, to determine if disease progression can be significantly slowed.

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: Perform a systematic examination of IL6 transsignaling in both initiation and progression of ALS.

During the past year, we have focused efforts on Aim 2 to generate the IL6R mouse model that exhibits enhanced shedding of the receptor. The breeding strategy with the SOD1 and IL6R^{TMD} mice will generate all mice required for characterization of IL6 transsignaling proposed in Aims 1 and 2.

Aim 2. Create an ALS model where IL6 transsignaling is increased and determine if disease pathology is altered.

2a. Progress on Mouse Model Construction

Before the initiation of grant funding, Dr. Hawkins (PI) and Dr. Milligan (Co-PI) met with Dr. Dale Cowley, Director, UNC Animal Models Core on 6/5/2018 at UNC Chapel Hill. During the review of our design of the IL6 transsignaling mouse, Dr. Cowley identified a potential issue with developing C57BL/6 *Il6ra*^{Sec}.

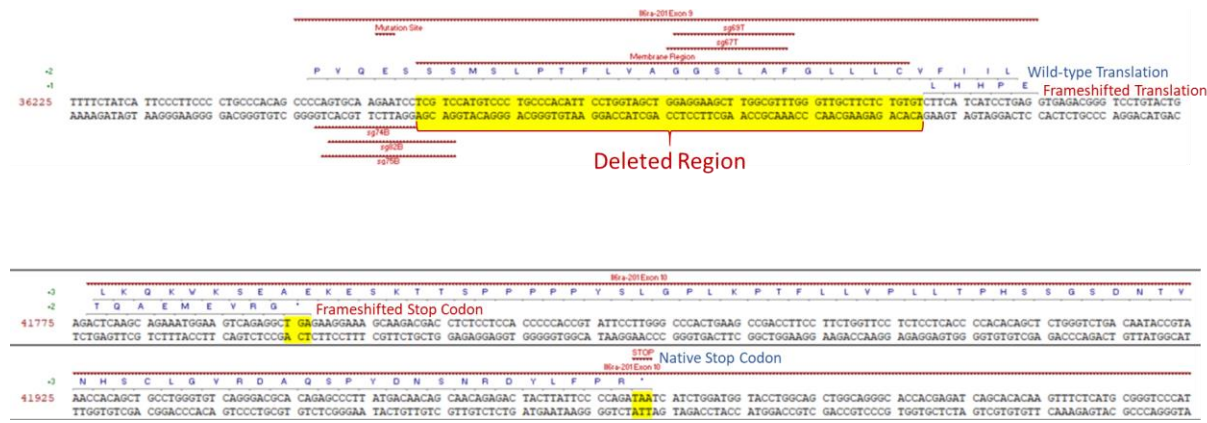
Based on this new design, a quotation for \$25,000 was obtained on June 11th, 2018 to construct this mouse.

Figure 1. Similarity of Human and Mouse IL6 Receptor Peptide

	Extracellular	Intracellular
Human	ATS*L*PVQDSSSVLPFLVAGGSLAFGLTLLCIAIVLRFKKTWKLRLKEGK	
Mouse	ATSVLAPVQESSMSLPTFLVAGGSLAFGLLLCVFIILRLKQKWKSEAEKESK	

^ (---Membrane region---)

Figure 1b. Mouse *Il6ra*^{Sec} CRISPR design sequence



2b. Timeline for Construction and Deliver of *Il6ra*^{Sec} Mice to WFUHS:

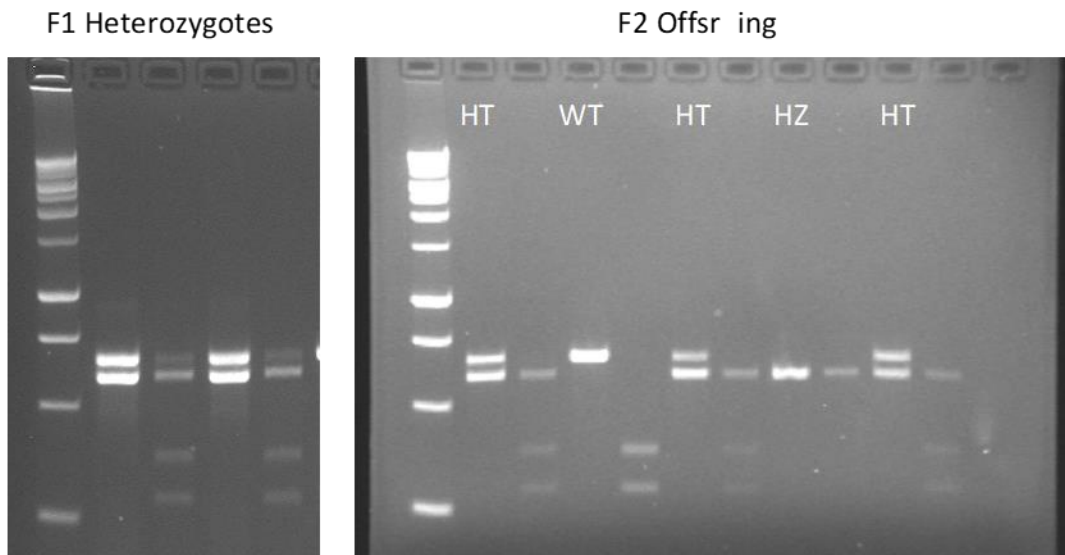
- August 2018, construction of the CRISPR initiated
- October 2018: construction of CRISPR mouse completed
- December 2018: Breeding lines completed
- January 22, 2019: Wake Forest Health Sciences Animal Resources lab received 2 breeding line and mice placed in quarantine.
 - Line 2; 6 females and 1 male
 - Line 5; 2 males
- Mice removed from quarantine (2/27/19)

2c. Genotyping of *Il6ra*^{Sec} Mice Based on the construction scheme, the transmembrane deletion created a secondary band when a PCR amplified target was analyzed by agarose gel electrophoresis. Figure 2 shows 2 *Il6ra*^{Sec} females from line 2 that are positive heterozygous carriers of the mutation (Lanes 2 and 4). A Hind III restriction site was also present in the sequence, and a digestion was performed to further confirm the construct. A male in line 5 produced an aberrant sized banding pattern and not used for further experiments. Sanger sequencing of all of the mice confirmed the proper transmembrane deletion in the sequence.

Figure 2. Left panel: PCR results from F1 Heterozygotes received from UNC Animal Model Core. Shown from left to right are ladder, PCR products of F1 female heterozygotes (upper band full length IL6R, lower

band IL6R with transmembrane deletion), Hind III digest, F1 male heterozygote. Right panel: PCR products of off-spring of F1 breeding pair. Expected genotypes are produced.

Figure 2.



As shown in Figure 2, the expected F2 genotypes are produced, in expected ratios from the F1 matings. We are continuing to breed the animals to generate sufficient numbers to begin to characterize expression levels of IL6R in serum, CSF, and tissues. We expect increased expression reflective of enhanced shedding in animals with IL6R transmembrane deletion. Initial characterization is expected to be complete by the end of summer. Once we have confirmed enhanced shedding of the IL6 receptor, we will initiate breeding with the SOD1 mouse model of ALS.

2d. Breeding of Mice

1e. Assessing sIL6r serum and CSF levels

We are currently assessing the levels of sIL6r in serum and CSF.

Aim 3. Treat the mouse models with transsignaling inhibitor, soluble gp130 to determine if disease progression can be significantly slowed

This aim will be completed once SOD1/IL6R^{Sec} mice are generated and characterized

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.

Our plans for the next reporting period are:

- 1. Finish validation of the *Il6ra^{Sec}* mouse model**
- 2. Breed the *Il6ra^{Sec}* mouse model with SOD1 mutant mice to establish the ALS/enhanced IL6 transsignaling model.**
- 3. Perform a systematic evaluation of IL6 transsignaling in both initiation and in progression of ALS in off spring of the *Il6ra^{Sec}*X SOD1 mice that will include SOD1, SOD1/*Il6ra^{Sec}* and *Il6ra^{Sec}* mice to complete experiments proposed in Aims 1 and 2.**
- 4. Treat the ALS/transsignaling mouse model with the transsignaling inhibitor, soluble gp130, to determine if disease progression can be significantly slowed.**

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).

By determining if IL6 transsignaling is critical in promoting ALS progression and severity, we will have identified a critical pathway for ALS treatment. The success of this study will also give us new insights into how the inflammation, and most specifically IL6 signaling, may contribute to ALS initiation.

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.

Nothing to report**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.

Nothing to report

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.

Nothing to report

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. Describe the technologies or techniques were shared.

We have developed a mouse model where the transmembrane domain of the IL6 receptor has been removed.

- **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.

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;*
- *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.*

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: Gregory Hawkins

Project Role: PI

Nearest person month worked: 2.7 months

Contribution to Project: Dr. Hawkins is a Professor of Biochemistry with experience in IL6 transsignaling research. Dr. Hawkins is working closely with Dr. Milligan in designing and testing the mouse model produced in this proposal and will be involved in data interpretation and manuscript preparation.

Funding support:

R03 AI37866-01

Generation of a mouse model to study IL6 transsignaling

This project will develop a transgenic mouse model to study IL6 transsignaling in multiple diseases.

Role: Co-Principal Investigator

P30 CA012197-43

Wake Forest Baptist Comprehensive Cancer Center - Cancer Center Support Grant

The Wake Forest Baptist Comprehensive Cancer Center is a multidisciplinary interdepartmental research center, organized into four divisions: Two Basic Science Programs, Clinical Research, and Cancer Prevention and Control.

Role: Co-Investigator

1R01HL122393-01A1

Social Stress, Diet, and Primate Monocyte Programing in Cardiovascular Risk

The project seeks to determine the effects of psychosocial stress on monocytes (cells that are important in the process of cardiovascular disease), and to evaluate whether a more healthy diet can improve the hypothesized adverse effects of psychosocial stress on monocytic characteristics.

Role: Co-Investigator

R01 NS036695-15A1

Genetic Environmental Risk Factors for Hemorrhagic Stroke

To determine the gene expression and epigenetic factors that contribute to hemorrhagic stroke.

Role: Co-investigator

DOD

Synergistic targeting of the JAK2-STAT3 and SMO-GLI1 pathways in triple-negative and HER2-enriched breast cancers

The primary objective of our application is to provide preclinical results as the basis for future clinical development of novel targeted combination therapies for triple-negative and trastuzumab-resistant HER2-positive breast cancers. The secondary objective is to move quickly towards clinical trials and thus maximize the translational impact of our proposal by concentrating our efforts on drugs that have received FDA approval for other diseases and/or are currently tested in clinical trials for breast cancer.

Role: Co-Investigator

1 R21 CA229027-01

Predicting Tumor Heterogeneity Evolution After Therapy in Patient-Derived Ex Vivo Glioblastoma Organoids

Glioblastoma (GBM) is a lethal, incurable form of cancer in the brain that universally recurs more aggressively even with maximally aggressive surgery followed by chemoradiotherapy. These tumors are extremely heterogeneous with regions of genetically distinct subclones that evolve differently over time and in response to treatments making designing effective therapies for each individual patient difficult. Here we propose to deploy a patient-specific ex vivo tumor-on-a-chip system to analyze tumor heterogeneity and drift over time to predict clonal evolution for patients, which could subsequently have a substantial impact on treatment decisions.

Role: Co-Investigator

1R01HL142992-01

Effects of Rare Variants and Ancestry on Beta Agonist Response in Asthma and COPD

Surveillance trials suggest that the risk for life-threatening asthma exacerbations and asthma-related deaths are increased with long-acting beta₂-adrenergic receptor (β₂AR) agonist (LABA) therapy; however, large clinical safety trials have not confirmed these observations despite studies showing that African Americans with asthma are more likely to respond adversely to LABA therapy. We have shown that ancestry-specific rare variants in the β₂AR gene are associated with worse asthma control in people using LABA and that African genetic ancestry associates strongly with lung function in African Americans with severe asthma and COPD suggesting that genetic variants could play a role in drug response and disease severity. We propose genetic studies based on β₂AR pathway gene variants and whole-genome studies of rare variants and genetic ancestry to identify novel mechanisms for inter-ethnic differences in drug response and disease severity.

Role: Co-Investigator

Name: Carol Milligan, PhD

Project Role: co-I

Nearest person month worked: 2.7 months on AL170130

Contribution to Project: Dr. Milligan is a Professor in Neurobiology and Anatomy with experience and expertise in neurodegenerative processes, notably those that occur in ALS. She is working with Dr. Hawkins in designing and testing the mouse model produced in this proposal, evaluating the role of IL6 transsignaling in the ALS mouse model and will be involved in data interpretation and manuscript preparation.

Funding support:

1 R03 AI137866-01

Development of IL6 Trans-signaling Mouse Model as a Shared Resource

This project is to develop a mouse model that incorporates a single nucleotide mutation in the protease cleavage site for the IL6 receptor that should mimic the human polymorphism that results in enhanced shedding of the receptor from the cell membrane. If successful, the model will be used in research related to neurodegenerative diseases, asthma, cancer, and cardiovascular diseases.

Role: co-PI

Overlap: None

NIH 5R25NS089458

Training in Health Disparity Research for a Diverse Neuroscience Workforce

This is a new Master's Program specifically developed to broaden educational opportunities for individuals interested in pursuing a career in Health Disparities in Neuroscience-related Disorders (HDND). The overall goal is to train individuals who will contribute to expanding diversity in the Neuroscience work-force. The program takes advantage of a strong Neuroscience Graduate Program and the Maya Angelou Center for Health Equity (MACHE) at WFU. Drs. Milligan, Director of the Neuroscience Program and Ronny Bell, Director of the MACHE are co-directors/co-PIs of the HDND MS program. Dr Berton took over for Dr. Bell when Dr. Bell left the institution.

Role: co-PI

Overlap: None

Hope for Tomorrow ALS Foundation (Milligan)

The funds provided support for preliminary studies of IL-6 signaling in ALS patients.

Role: PI (effort as needed)

Overlap: None

Brian White ALS Foundation Funds

These funds are a contribution by the Department of Neurology for the Project "Hsp70 as a Potential Treatment for ALS"

Role: PI (effort as needed)

Overlap: None

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.

Nothing to report

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.*

Nothing to report