AWARD NUMBER: W81XWH-15-1-0437

TITLE: Biomarkers and Brain Mechanisms of Gulf War Illness

PRINCIPAL INVESTIGATOR: Dikoma C. Shungu, Ph.D.

REPORT DATE: Sept 2021

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**
Sept 2021	Annual	09/01/2020-08/31/2021

4. TITLE AND SUBTITLE

Biomarkers and Brain Mechanisms of Gulf War Illness

5a. CONTRACT NUMBER
W81XWH-15-1-0437

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dikoma C. Shungu, Ph.D.

E-Mail : dcs7001@med.cornell.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Weill Cornell Medicine
1300 York Ave
NEW YORK NY 10065-4805

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: [**no new findings; original abstract provided**] Gulf War illness (GWI), a chronic and debilitating pain, headaches, impaired memory and thinking, fatigue, respiratory and gastrointestinal symptoms, and skin abnormalities. Exposure and sensitivity to chemical, pharmaceutical and/or environmental toxins in a combat theater of operations is believed to be causative of the illness. The pathobiological mechanisms of GWI are unknown; there are no validated diagnostic tests, nor are there effective treatments or cures. This is a case-control study consisting of 20 Gulf War veterans affected with GWI and 20 matched non-affected Gulf War veterans, who will serve as the normal control group. All subjects will undergo brain positron emission tomography and magnetic resonance imaging scans for assessments of metabolic or neurochemical disturbances that may be associated with GWI. In all consenting participants, a lumbar puncture will be performed to obtain cerebrospinal fluid (CSF), which will be analyzed for abnormalities in biochemical compounds that may be related to GWI. The derived neuroimaging and CSF metabolic or biochemical data will be compared between the groups to determine if there are abnormal changes in GWI veterans compared to controls, which may shed new light onto the pathophysiology of GWI, as well as serve as biomarkers of the disorder.

15. SUBJECT TERMS
Gulf War illness, neuroinflammation, oxidative stress, mitochondrial dysfunction, magnetic resonance imaging (MRI), Positron Emission Tomography (PET)

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	19	USAMRDC

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

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

The overall objective of this study is to evaluate the suitability of a number of endogenous chemical compounds or metabolites to serve as sensitive brain imaging and cerebrospinal fluid (CSF) biomarkers of pathologic alterations in Gulf War illness (GWI) for use to facilitate early diagnosis, to assess disease progression and/or to monitor therapeutic response in future clinical trials of promising interventions. This is a case-control study that will enroll 20 Gulf War veterans affected with GWI and 20 matched non-affected Gulf War veterans, who will serve as the normal control group. All subjects will undergo brain positron emission tomography (PET) and magnetic resonance imaging (MRI) and spectroscopy (MRS) scans to assess metabolic or neurochemical disturbances that may be associated with GWI. In all consenting participants, a lumbar puncture will be performed to obtain CSF samples, which will be analyzed for abnormalities in biochemical compounds that may be related to GWI. The derived neuroimaging and CSF metabolic or biochemical data will be compared between the groups to determine if there are abnormal changes in GWI veterans compared to controls, which may shed new light onto the pathophysiology of GWI, as well as serve as biomarkers of the disorder.

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

Gulf War illness, Neuroinflammation, Oxidative Stress, Mitochondrial Dysfunction, Magnetic Resonance Imaging, Magnetic Resonance Spectroscopy, Positron Emission Tomography.

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.

RESEARCH-SPECIFIC TASKS:		
For All Specific Aims: Recruitment & Regulatory Approvals	**Timeline**	**Site(s)**
Major Task 1: GWI and Non-GWI Subject Recruitment	Months	ALL
Subtask 1: Establish formal contact between Mount Sinai Beth Israel (MSBI) Medical Center and the New Jersey War Related Illness & Injury Study Center [NJ WRIISC] to discuss strategy for recruiting GWI and non-GWI veterans for the study.	1	Dr. Natelson & Dr. Helmer

Subtask 2: Develop complementary or cooperative IRB protocols, including study advertisement material that would enable seamless recruitment/characterization of subjects at NJ WRIISC/MSBI and referral to Weill Cornell Medicine [WCM].	1-3	Drs. Natelson, Helmer & Shungu
Subtask 3: Submit IRB protocols at each participating Institution. **Second-tier DoD human subjects regulatory review and approval conducted by the Office of Research Protections, Human Research Protections Office (HRPO)**.	**3-6**	Drs. Natelson, Helmer & Shungu; HRPO
Milestone(s) Achieved: All IRB protocols approved; recruitment starts in earnest by month 6 and will continue to end of project	6-30	Drs. Natelson, Helmer
Specific Aim 1: Neuroimaging Biomarkers Studies		
Major Task 2: Conduct *in vivo* brain ^{11}C-(R)-PK11195 PET to assess **neuroinflammation**		WCM
Subtask 1: Order supply for producing the radioligand and review chemistry and PET scanning protocol.	1-6	Dr. Babich
Subtask 2: Conduct PET scans in 10 GWI and 10 non-GWI veterans	6-30	Dr. Babich
Milestone(s) Achieved: Availability of radioligand on demand to end of study; clear ability to obtain good PET scans, reproducibly, in each subject using the PK11195 PET technique	30	
Major Task 3: To conduct ^{1}H and ^{31}P MRS studies for assessment of **oxidative stress** and **mitochondrial dysfunction** *in vivo*. Assess cerebral blood flow using ASL-MRI.		WCM
Subtask 1: Protocols for achievement of this Major Task are already fully developed and being used in an ongoing study in chronic fatigue syndrome that is identical to the one we are proposing in GWI. The protocol will be reviewed with the MR neuroimaging team to ensure its flawless implementation.	1-6	Dr. Shungu
Subtask 2: Conduct ^{1}H and ^{31}P MRS and scan in 20 GWI and 20 non-GWI veterans to assess oxidative stress and mitochondrial dysfunction; also measure CBF in all 40 subjects using ASL-MRI.	6-30	Dr. Shungu
Milestone(s) Achieved: Clear ability to obtain high-quality ^{1}H and ^{31}P MR spectra, as well as ASL-MRI CBF maps in each enrolled subject.	30	

Specific Aim 2: CSF Biomarkers		
Major Task 4: Collect CSF samples from all consenting subjects for validation of neuroimaging biomarkers.		WCM
Subtask 1: Collect and cryo-freeze CSF samples using lumbar puncture	6-30	Dr. Mangat
*Milestone(s) Achieved: Clear ability to collect and freeze CSF samples for later analyses to determine markers of oxidative stress and neuroinflammation (cytokines, **including IL-17**).*	30	
For Specific Aims 1 & 2: Data Analysis and Hypothesis Testing		
Major Task 5: **Data Analysis, Reduction, Statistical Analyses**.		WCM
Subtask 1: Analyze/process and reduce the data from all the active tasks, combine with the clinical data in a **master** database and perform statistical analyses and hypothesis testing.	30-36	All investigators with Dr. Shungu supervising
*Milestone(s) Achieved: Determination of whether: (a) **neuroinflammation**, **oxidative stress** and **mitochondrial dysfunction** play a role in GWI pathobiology; and (b) the outcome measures either individually or in concert can serve as **biomarkers** for GWI and point to potential **brain mechanisms** for the illness.* *Submission of at least 3 manuscripts for publication.*	36	

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.

ACCOMLISHMENT & FINAL PROGRESS REPORT FOR FUNDING PERIOD 09/01/2015 TO 8/31/2021

1. Overview and Recapitulation of the Proposed Research Objectives

The aims of the present study were: (a) to use in vivo brain 11C-(R)-PK11195 positron emission tomography (PET) to measure the binding potential of the ligand as a marker of neuroinflammation; (b) to use proton magnetic resonance spectroscopy (^{1}H MRS) to measure in vivo brain levels of glutathione (GSH) -- the most abundant antioxidant in the central nervous system – as a marker of oxidative stress; (c) to use 1H MRS to measure in vivo brain levels of lactate and N-acetylaspartate (NAA) as markers of mitochondrial dysfunction; (d) to use ^{31}P MRS to measure in vivo brain levels of ATP, creatine phosphate (PCr) and inorganic phosphate (Pi) as complementary indices of mitochondrial dysfunction, and phosphomonoesters and phosphodiesters as indices of lipid peroxidation and oxidative stress; and (e) to measure cerebral blood flow with arterial spin-labeling MRI to assess whether hypoperfusion is also implicated. In addition, to validate the preceding neuroimaging measures, markers of neuroinflammation and oxidative stress will also be measured in the cerebrospinal fluid (CSF) samples obtained by lumbar puncture in consenting participants using standard biochemical assays. A case-control study design consisting of 20 veterans affected with GWI and of a group of 20 matched non-affected Gulf veterans who served as the normal control group. All the subjects underwent the neuroimaging scans, bodily fluids collection (urine, plasma and CSF samples from those who consented to provide CSF samples. The derived neuroimaging and metabolic data from bodily fluids compared between the groups to determine if there are abnormal changes in the GWI veterans compared to controls. To test the hypothesis, with a multimodal experimental approach, that neuroinflammation, oxidative stress and mitochondrial dysfunction play pathogenic roles in the illness.

2. Enrollment

For this study, we had aimed for **target enrollments** of 20 veterans affected with GWI and of a group of 20 matched non-affected Gulf veterans who served as the normal control group. During our 3rd NCE request, this target enrollment had been revised and approved by USAMRAA Grant & Contract leadership to enrollment 30 subjects, consisting of 15 patients with Gulf War Illness (GWI) and 15 healthy Gulf War veterans.

After we fulfilled all regulatory requirements (Local IRB approvals and initial USAMRMC HRPO approval on 05/02/2016), Participant recruitment began in May 2016 and ended in August 2021. A total of 28 veterans (15 GWI and 13 HV) met the eligibility criteria for participation and consented to enter the study. Each enrolled subject underwent all components of assessments. As of the end of the study on 8/31/2021, our **actual or final enrollment** consisted of 15 veterans with GWI and 13 Healthy veterans, who had successfully completed the various components of the study assessments (**Table 1**).

Table 1: Actual and Target Enrollments for Reporting Period (09/01/2015-08/31/2021)

Diagnostic Group	Target Enrollments	Actual Enrollments
Veterans with GWI	**15**	**15**
Healthy Veterans	**15**	**13**
Total	**30**	**28**

The demographic and ethnic breakdown of all the enrolled subjects is provided in the attached Cumulative Inclusion Enrollment Report. Table 2, titled Inclusion Enrollment report, provides the demographics and ethnic breakdown of the 28 subjects:

Table 2 Inclusion Enrollment Report

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race				
Ethnic Category	**Females**	**Males**	**Sex/Gender Unknown or Not Reported**	**Total**
Hispanic or Latino	0	5	0	5 **
Not Hispanic or Latino	2	21	0	23
Unknown (individuals not reporting ethnicity)	0	0	0	0
Ethnic Category: Total of All Subjects*	2	26	0	28 *
Racial Categories				
American Indian/Alaska Native	0	0	0	0
Asian	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0
Black or African American	0	7	0	7
White	2	14	0	16
More Than One Race	0	0	0	0
Unknown or Not Reported	0	0	0	0
Racial Categories: Total of All Subjects*	2	21	0	23 *
PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)				
Racial Categories	**Females**	**Males**	**Sex/Gender Unknown or Not Reported**	**Total**
American Indian or Alaska Native	0	0	0	0
Asian	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0
Black or African American	0	1	0	1
White	0	3	0	3
More Than One Race	0	1	0	1
Unknown or Not Reported	0	0	0	0

3. Study Assessments

Clinical Assessment Measures:

All 28 subjects completed all the clinical assessments. Clinical characteristics as assessed by a battery of standardized tests of overall health and functional disability consisted of:

1. The CDC CFS Symptom Inventory: CDC CFS Symptom Inventory is a 19-item self-report that measures the frequency and intensity of symptoms related to CFS, including post-exertional fatigue, sleep disturbance, problems remembering or concentrating, muscle aches and pains, joint pain, sore throat, tender lymph nodes and swollen glands, and headaches. The total possible score ranges from 0 to 304, with higher scores indicating greater severity.
2. Multidimensional Fatigue Inventory (MFI): MFI is a 20-item self-report designed to measure fatigue along the following dimensions: General Fatigue, Physical Fatigue, Mental Fatigue, Reduced Motivation and Reduced Activity. Each item is rated from 0 to 5 yielding a possible score range of 0 to 100, with higher scores indicating greater severity.
3 Inventory of Depressive symptomatology (IDS-SR): IDS-SR is a validated instrument designed to assess the severity of depressive symptoms. The scale is scored by summing responses to 28 of the 30 items to obtain a total score ranging from 0 to 84, with higher scores indicated greater severity of depression.
4. RAND: The RAND 36-Item Health Survey taps eight health concepts: physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions.

Neuroimaging**:**

MR scans: MRS scans to derive our primary indices of CNS oxidative stress and mitochondrial dysfunction had been successfully performed in the enrolled subjects. The outcome measures consisted of: (a) in vivo brain levels of glutathione (GSH) as a marker of oxidative stress and (b) in vivo levels of ventricular lactate and cortical N-acetylaspartate (NAA) as markers of mitochondrial dysfunction measured with 1H MRS, and (c) in vivo brain levels of ATP, creatine phosphate (PCr) and inorganic phosphate (Pi) also as indices of mitochondrial dysfunction measured with ^{31}P MRS imaging.

PET scans: in vivo CNS neuroinflammation has been assessed in all subjects through measurement of the binding potential of the radioligand 11C-(R)-PK11195 using positron emission tomography (PET).

Body Fluid Samples:

The following body fluid samples have been collected in the indicated subjects and then stored at -80 ^{o}C:

1. Plasma – to derive peripheral markers of oxidative stress (all subjects)
2. Urine – to derive peripheral markers of oxidative stress (all subjects)
3. CSF – to derive central markers of oxidative stress (consenting subjects only)

Table 3: Number of subjects per Study Assessment

Group	No. of Clinical Assessments	No. of MR Scans	No. of PET Scans	No. of plasma samples	No. of urine samples	No. of CSF samples
Veterans with GWI	15	14	15	15	15	0
Healthy Veterans	13	13	7	13	13	0
Total	28	27	22	28	28	0

Note on the CSF Samples: To provide CSF sample subjects had to undergo a lumbar puncture. However, the procedure is not without discomfort, so we discontinued it after most participants opted out.

4. SPECIFIC AIMS AND HYPOTHESES AND ACCOMPLISHMENTS:

The overall hypothesis of this GWIRP-NIA project was that neuroinflammation, oxidative stress and mitochondrial dysfunction play pathogenic roles in GWI, as postulated in our working model. To test this overall hypothesis, we proposed the following Specific Aims and associated Hypotheses for this project:

4.1 Specific Aim 1 *In Vivo* Neuroimaging Biomarkers:

In veterans with GWI and in a matched non-affected veterans (a) to use in vivo brain 11C-(R)-PK11195 PET to measure the binding potential of the ligand as a marker of neuroinflammation; (b) to use 1H MRS to measure in vivo brain levels of glutathione (GSH) as a marker of oxidative stress; (c) to use ^{1}H MRSI to measure in vivo brain levels of lactate and NAA as markers of mitochondrial dysfunction; (d) to use ^{31}P MRSI to measure in vivo brain levels of ATP, PCr and Pi as complementary indices of mitochondrial dysfunction, and the phosphomonoesters and phosphodiesters as indices of lipid peroxidation and oxidative stress; and (e) to use ASL-MRI to measure rCBF to sort out between hypoperfusion and mitochondrial dysfunction as a cause for increased brain lactate, if present.

Hypothesis 1: Based on our working model, we hypothesize that veterans with GWI will show abnormalities in a number of the proposed neuroimaging measures, which may serve as biomarkers of the illness, point to potential brain mechanisms, and inform future larger studies.

All neuroimaging data are currently being analyzed.

4.2 Specific Aim 2 *In Vitro* Biomarker Validation:

To measure temporally concordant levels of a number of established markers of neuroinflammation (cytokines) and oxidative stress (GSH, isoprostanes) in CSF samples obtained from consenting participants.
Hypothesis 2: The derived CSF metabolite profile will complement the neuroimaging biomarkers, and if correlated with the latter, would achieve cross-validation and thus serve as less expensive and more clinically accessible biomarkers of GWI.

Because most participants opted out of undergoing lumbar puncture that is required to collect CSF samples, a decision was made to discontinue this Specific Aim.

Hypothesis 3 Brain Mechanisms: The complementary neuroimaging biomarkers will contribute novel and direct information on the pathobiological abnormalities that underlie GWI, which, through cross-correlation and corroboration with biochemical data derived from the CSF samples could point to brain mechanisms that underpin the illness.
Hypothesis 4 Clinical Correlations: The proposed neuroimaging and CSF outcome measures (collectively referred to as "objective outcome measures") will correlate with clinical characteristics and manifestations of GWI as assessed by a battery of standardized tests of overall health and functional disability.

5. ACCOMPLISHMENTS:

Data analyses and reduction and statistical analyses are currently in progress.

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.

Nothing to Report

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).

Several aspects of this study may have an impact on GWI research and our understanding of the condition, they represented a departure from nearly all the studies that have been conducted in the disorder to date:

1. Multimodal Experimental Approach: This study brought together a strong multidisciplinary team of investigators, with expertise in neuroimaging and in GWI and related disorders (chronic fatigue syndrome and fibromyalgia) to conduct what may have been the first comprehensive and multimodal attempt at developing and validating a number of highly promising biomarkers of GWI, based on measuring, all at once, multiple objective outcome measures that reflect the metabolic, physiologic and clinical abnormalities that we postulate are manifestations of the biological dysfunctions in GWI. Potential benefits of the proposed approach include the opportunity to (a) derive novel information on alternate but likely complementary "biomarkers", (b) validate a combination of "biomarkers" that might be more useful for objective disease diagnosis, progression and therapeutic response monitoring and subtyping than a single marker, especially in light of clinical heterogeneity in the disorder, and (c) enable cross-validation through correlational analyses. Importantly, regardless of the ultimate outcome of this study, the multimodal neuroimaging study may produce such a wealth of metabolic, physiologic, biochemical and clinical data in GWI that would represent an important contribution to a disorder about which there is currently a paucity of objective biomedical data acquired simultaneously in the same cohorts.
2. Study was Mechanistic: This study was highly innovative in that it was almost entirely model-driven, drawing both in concept and in research strategy from studies in CFS, a closely related multi-symptom illness, with key points identified along a defined pathway, where objective measurements be made to test the validity of the model. Our multidisciplinary team of investigators worked synergistically to try to recruit well characterized GWI patients in whom we used state-of-the-art neuroimaging methods to derive objective indices of pathophysiological abnormalities that are postulated to underlie GWI. Thus, a potential impact of this research for GWI is the possibility to shed new light onto its pathobiology and underlying brain mechanism(s).
3. Study Offered the Possibility to Differentiate GWI from other Chronic Multi-symptom Illnesses: A central question and unmet need in the study of GWI and other closely related multi-symptom illnesses is whether and how these illnesses can be objectively differentiated from one another. While there is extensive symptom overlap among the various multi-symptom illnesses, there is ample evidence that each is a distinct medical entity, with a highly heterogeneous clinical presentation. Because the model that we proposed to investigate for GWI and the experimental approach that we used to try to validate this model were virtually identical to those that we had used and are currently using in our studies of CFS and FM, we will the opportunity to compare and try to differentiate GWI from CFS and FM based on the objective neuroimaging and clinical data obtained through this research project. In other words, our proposed research looks beyond a single multi-symptom illness. It pits them against each other in manner that may enable their objective differentiation, since we would be comparing identical objective biomarkers rather than clinical symptoms.

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.

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.

None to date.

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

None

Significant changes in use or care of vertebrate animals.

Not applicable

Significant changes in use of biohazards and/or select agents

Not applicable

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.*

In progress.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research

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."

FOR PERIOD 09/01/2015 TO 8/31/2021

Name: Dikoma C. Shungu, Ph.D.
Project Role: PI
Researcher Identifier (e.g. ORCID ID): orcid.org/0000-0001-9452-2245
Nearest person month worked: 1 calendar Month
Contribution to Project: Dr. Shungu oversees all the MR Neuroimaging aspects the proposed research, as well as its day-to-day coordination of the study.

Name: Xiangling Mao, M.S.
Project Role: Co-I
Researcher Identifier (e.g. ORCID ID): orcid.org/0000-0003-2274-8282
Nearest person month worked: 1 Calendar Month
Contribution to Project: Ms. Mao perform work in the area of regulatory activity, MR Scan and data processing.

FOR PERIOD 09/01/2015 TO 8/31/2018

Name: Yeona Kang, Ph.D.
Project Role: Co-I
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1 calendar Month
Contribution to Project: PET data processing

FOR PERIOD 09/01/2015 TO 8/31/2020

Name: Benjamin H. Natelson, M.D.
Project Role: Sub-site PI at BIMC
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1 Calendar Month
Contribution to Project: Dr. Natelson will perform work in the area of subject recruitment and characterization.

Name: Sarah Khan
Project Role: Study coordinator at BIMC
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1 Calendar Month
Contribution to Project: Ms. Khan will perform work in the area of subject recruitment.

Name: Diana Vu
Project Role: Study coordinator at BIMC
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1 Calendar Month
Contribution to Project: Ms. Khan will perform work in the area of subject recruitment.

Name: Michelle Blate
Project Role: Nurse-practitioner at MSBI
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1 Calendar Month
Contribution to Project: Ms. Blate performs work in the area of subject recruitment.

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 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.