AWARD NUMBER: W81XWH-17-2-0019

TITLE: Assessment of the Immediate, Short- and Long-Term Deleterious Consequences of Polytraumatic Injury in a Critical Care Non-Human Primate Model

PRINCIPAL INVESTIGATOR: Dr. Matthew Bradley

RECIPIENT: The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.

REPORT DATE: JULY 2020

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
JULY 2020

2. REPORT TYPE Annual

3. DATES COVERED
01 July 2019 – 30 June 2020

4. TITLE AND SUBTITLE Assessment of the Immediate, Short- and Long-Term Deleterious Consequences of Polytraumatic Injury in a Critical Care Non-Human Primate Model

5a. CONTRACT NUMBER
W81XWH-17-2-0019

5b. GRANT NUMBER
W81XWH-17-2-0019

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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8. PERFORMING ORGANIZATION REPORT NUMBER

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
Multi-organ failure (MOF) remains a significant cause of morbidity and mortality in trauma. The pathophysiology behind MOF is related to a maladaptive systemic inflammatory response syndrome (SIRS) triggered by the release of excessive inflammatory cytokines. Currently, the underlying mechanism for immune dysregulation is poorly understood, and there are no existing pre-clinical models, which accurately reflect the clinical scenarios that play out in severely injured patients prone to development of SIRS. The study will fill existing and emerging gaps in the Combat Casualty Care Program to improve treatment for service members injured in combat. Understanding and defining the physiologic and immunologic responses after polytraumatic injury in a stringent and relevant animal model will provide insight for the clinical management and for the development of innovative therapeutic interventions/strategies designed to improve the clinical outcome after combat-related trauma. The goal is to fully characterize the immune response in a clinically-relevant NHP trauma model in anticipation of identifying therapeutic targets to mitigate the associated SIRS/MOF following poly-traumatic injury.

15. SUBJECT TERMS
Trauma, non-human primate, systemic inflammatory response, multiple organ failure, animal models, inflammation, wound healing

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	16	**19b. TELEPHONE NUMBER** *(include area code)*

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.

Multi-organ failure (MOF) remains a significant cause of morbidity and mortality in trauma. The pathophysiology behind MOF is related to a maladaptive systemic inflammatory response syndrome (SIRS) triggered by the release of excessive inflammatory cytokines. Currently, the underlying mechanism for immune dysregulation is poorly understood, and there are no existing pre-clinical models, which accurately reflect the clinical scenarios that play out in severely injured patients prone to development of SIRS. Further, outside of supportive care, there are limited existing strategies for preventing or treating SIRS following complex combat trauma. Moreover, this threatening immunological phenomenon has not been systematically studied as a potential important contributing risk factor for post-injury health complications. In this proposal, we are refining our non-human primate (NHP) poly-trauma model using prolonged ventilation and intensive care unit environment. The goal is to fully characterize the immune response in a clinically-relevant NHP trauma model in anticipation of identifying therapeutic targets to mitigate the associated SIRS/MOF following poly-traumatic injury.

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

Trauma, non-human primate, systemic inflammatory response, multiple organ failure, animal models, inflammation, wound healing

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.

Regulatory Approvals (Milestones): Local BUMED/ACURO/IACUC approval. CRADA amended. Approval of purchase contract for NHPs.

We completed our Regulatory Approvals milestones after receiving local BUMED/ACURO/IACUC approval, CRADA amendment, and approval of purchase contract for NHPs on 24-NOV-2017.

Specific Aim 1 Major Task 1 (Milestones): Completion of animal procurements, ICU facility, pre-experimental health monitoring of NHPs, recruitment/training of additional essential surgical and post-operative care staff.

Specific Aim 1, Major Task 1 completed. We procured animals, developed an ICU for care of NHPs on 26-FEB-2018, performed pre-experimental health monitoring of NHPs, and recruited/trained additional essential surgical and post-operative care staff on 05-FEB-2018.

Specific Aim 1 Major Task 2

Subtask 1: Polytraumatic injury studies with short-term ventilator support in an ICU setting.

Subtask 2: Sample collections and processing to include baseline measurements through a 6-month post-operative time point. (Milestones): Completion of 6-month follow-on clinical assessments and sample collections

We successfully performed a pilot study of maintaining sedation and ventilation of three NHPs for 72 hours at the request of the IACUC on 19Mar-2018 and 2-APR-2018. In the upcoming year we will begin the polytrauma model with ventilatory and intensive care unit management.

Specific Aim 2 Major Task 1 (0% completed)

Subtask 1: Assessment of short-term and long-term co-morbidities and survival (mortality). Determine the impact of mechanical ventilations.

Subtask 2: Clinical and molecular assessment of SIRS, ARDs, and MODS/MOF induction and organ system functional recovery kinetics.

Subtask 3: Assessment of systematic inflammatory, hemodynamic, metabolic, and trauma-induced immune cell activation responses, coagulation pathway activation, post-injury wound repair/healing. (Milestones): Completion of in vivo experiments and clinical assessment studies and data analysis

Specific Aim 2 Major Task 2 (0% completed)

Subtask 1: Determine the long-term pathophysiology of polytraumatic injury on the respiratory, cardiac, genitourinary, gastrointestinal, and innate plus adaptive immune system as well as skin and soft tissue wound reparative/healing/regenerative process. (Milestones): Completion of in vivo experiments and clinical assessment studies and data analysis.

Specific Aim 2 and its Major Tasks/Subtasks will be completed in the upcoming year. In discussion within our research group based on the pilot study and in collaboration with Veterinary Medicine and the IACUC, we determined that the model should be slightly modified to improve the assessment of morbidity (MOF), remove variability, and improve the fidelity of the model by removing the post-extubation portion of the model. Leaving the animal intubated for the entirety of the experiment and maintaining the NHP in an ICU setting will provide more robust, complete data and allow us to better assess multi-organ failure. Extubating the NHP induces variability of care and data collection inherent to non-intubated patients. Thus, animals will now be intubated for the entirety of the experiment (total 5 days) and then euthanized. As mentioned this will give a better assessment of the physiological and immunological response to trauma.

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.

Successfully completed a reproducible prolonged mechanical ventilation and sedation plan in pilots/sham NHPs with the ability to successfully awake and remove them from mechanical ventilation.

We requested and were granted a 1 year no cost extension.

Individual peripheral blood samples were collected from available NHPs and, using an antibody panel for Flow Cytometry, we screened T cell populations for immune activation state prior to enrolling the NHPs in the pilot study. We postulated that the pre-existing immune activation may unfavorably skew the results (baseline data collection).
Following our baseline characterization, we successfully completed a pilot series (on the recommendation of our Veterinarians and IACUC department) wherein we maintained three non-human primates intubated and sedated for three days. Figure 2 is a characterization of their peripheral blood cell profile over time while intubated to assess the effects of ventilation and sedation on these cell lines. Of note, there was an initial rise in Macrophages, NK cells and Neutrophils approximately two hours after intubation and sedation with a subsequent decrease in these cell lines back to baseline. Figure 3 demonstrates the trend in hemoglobin (Hgb) and creatinine over the study period. Over time there was a decrease in Hgb likely attributed to a dilutional effect from crystalloid infusions while the animal was intubated. Figure 4 is a profile of the heart rate (HR) and mean arterial pressure (MAP) over time. Overall, the HR was variable throughout the study but in general higher initially after intubation. Conversely, the MAP was lowest immediately after intubation likely related to the effects on anesthesia induction.

Results: As indicated in Fig.1, the NHPs enrolled in our protocol showed a range of baseline immune activation profiles. We used Flow Cytometry to screen and select NHPs with the lower levels of effector $CD4^+$ T cells ($CD45^+ CD3^+ CD4^+ CD28^{lo} CD95^{hi}$) vs. naïve $CD4^+$ T cells ($CD45^+ CD3^+ CD4^+ CD28^{hi} CD95^{lo}$) and effector $CD8^+$ T cells($CD45^+ CD3^+ CD8^+ CD28^{lo} CD95^{hi}$) vs. naïve $CD8^+$ T-cells (green boxed NHP subjects, graphs on the left, Fig.1), respectively. To highlight our rationale, a comparison of cells with a higher percentage of effector $CD4^+$ and $CD8^+$ T-cells (Animal 12U014 in red box in graphs on the left, Fig.1) to lower effector T cell percentages is provided as an example (Animal 13U026 in green box in graphs on the left, Fig. 1). The flow cytometry analysis panels (right panels, Fig.1) likewise demonstrate favorable (in green) and unfavorable (in red) distribution of peripheral cells in addition to highlighting the gating strategy. The distinction between favorable and unfavorable is made on the basis that resting state peripheral blood T cells should skew towards naïve first, then memory ($CD45^+ CD3^+ CD28^{hi} CD95^{hi}$), then effector last. A relatively higher percentage of effector cells is consistent with recent activation of the immune system. Additionally, we used published data on naïve/memory/effector phenotypes in comparably aged male Rhesus Macaques as a point of comparison in deriving favorable vs. unfavorable characterizations.

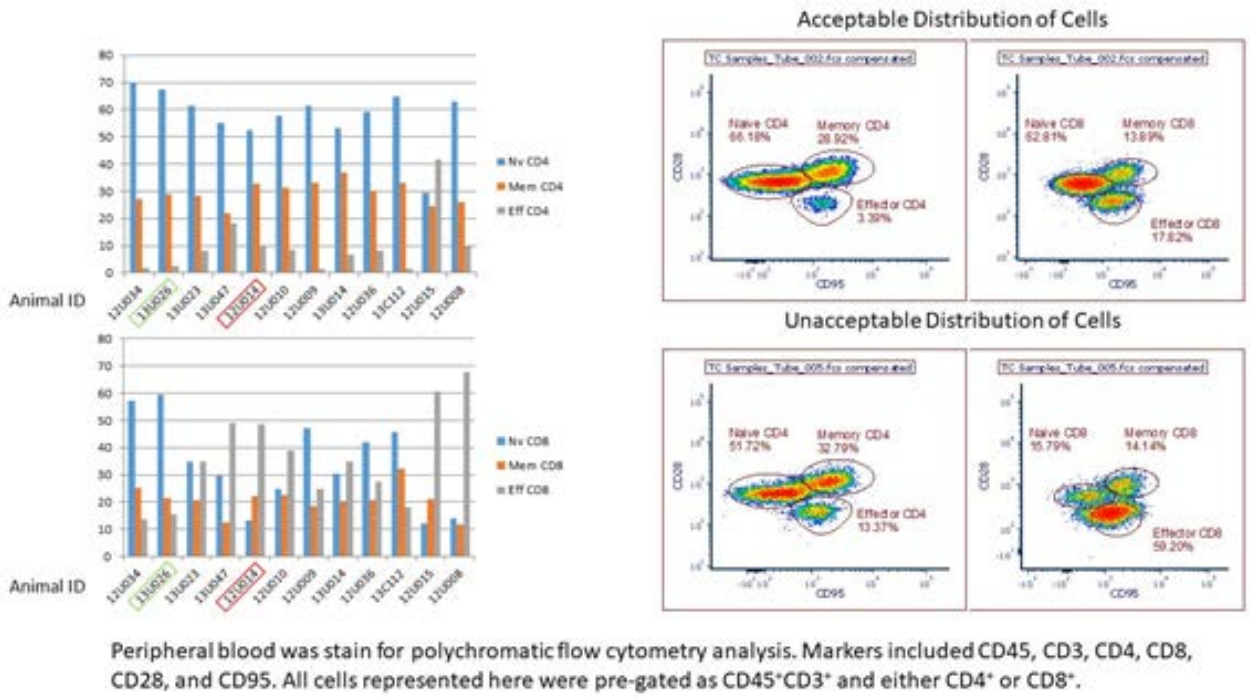

Fig.1 Screening peripheral blood cells from NHPs to establish a selection criteria to enroll animals in this study. Peripheral blood cells were stained for Polychromatic Flow Cytometry analysis. Markers included CD45, CD3, CD4, CD8, CD28, and CD95. All cells represented here were pre-gated as CD45+CD3+ and either CD4+ or CD8+. T cells may be divided in phenotypically unique subsets that include naïve, memory, and effector T cells, within both the CD4+ and CD8+ compartments. In Rhesus Macaques these populations can be distinguished based on the relative expression of CD28 and CD95 as either naïve ($CD28^+$ CD95-/Lo), memory ($CD28^+$ $CD95^+$), or effector ($CD28^-$ $CD95^+$) as indicated in the dot plot. A shift towards the effector compartment and away from the naïve compartment is consistent with an activated state resulting from antigen specific

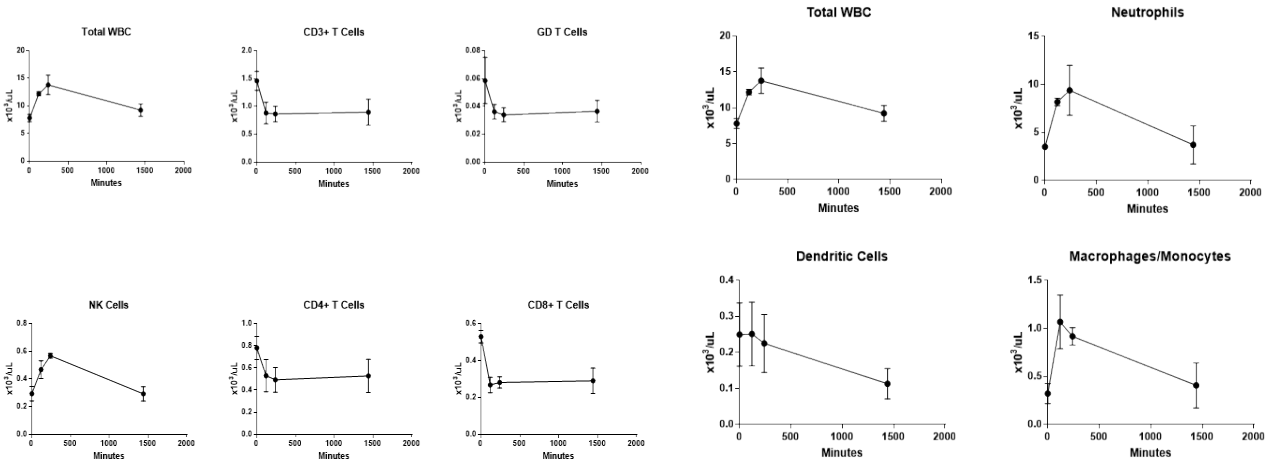

Figure 2. Characterization of peripheral blood cells in intubated NHPs. Peripheral blood was collected at the indicated time points into EDTA containing tubes. Staining for polychromatic flow cytometry analysis was performed using the following targets: TCR-gd, NKp80, CD3, CD28, CD45, CD95, CD4, CD8, MHC Class II, CD11b, CD11c, CD14, CD64, CD16, CD20, CD163, and Invitrogen Fixable Live/Dead. Quantification of cellular subsets was performed using CBC Leukocyte concentrations combined with the percentage of gated cells. Error bars display standard error of the mean (SEM).

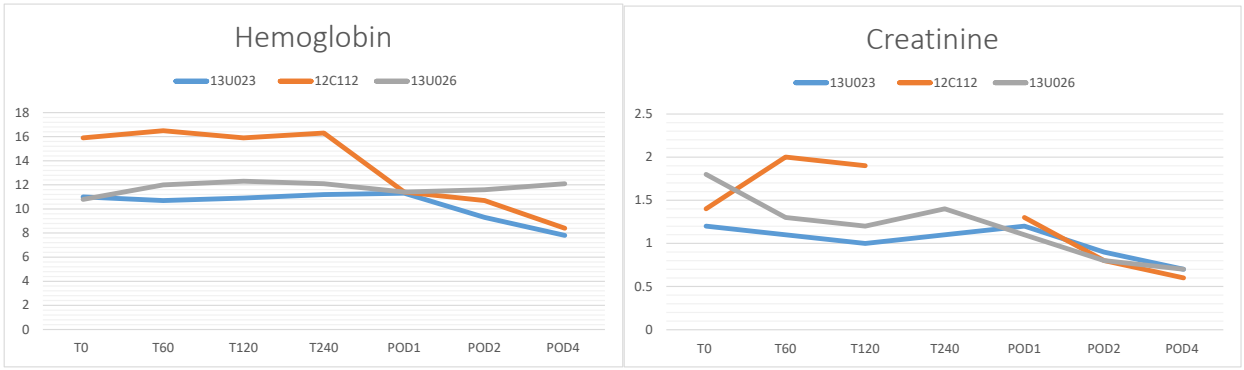

Figure 3. Trend of Hemoglobin and Creatinine over the study time period
Hemoglobin decreased in two NHPs over the study time period. Likewise, creatinine also trended down at POD 1. Both downward trends likely the result of the dilutional effect from fluid resuscitation.

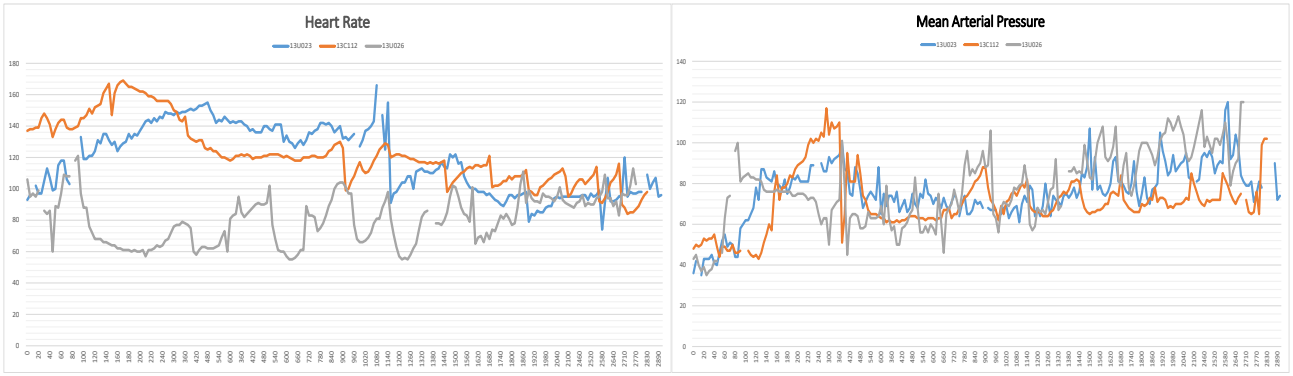

Figure 4. Trend of Heart Rate and Mean Arterial Pressure over the study time period
Heart rate tended to be higher at the beginning of the study while the arterial pressure trend increased over the captured time period.

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.

Provided training for one resident, Dr. Atwood for ICU care. He was able to perform and fine tune his skills on arterial and venous catheterization without any complications. The resident was able to perform laparoscopic procedures without any injuries to major organs.

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.

Due to the COVID-19 pandemic all non-COVID related research has been halted at WRAIR/NMRC since March 2020. During the next reporting period, we are planning to complete the animal experiments. NHPs are in-house for the next batch of experiments.

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

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."
Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report

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

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

Nothing to Report

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

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

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

Nothing to Report

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

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

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

Nothing to Report

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

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

- An additional amendment to modify the ICU portion of the study was denied by the IACUC and instead a request was made to submit a new protocol.
- A new protocol was submitted May 2019 and sent back for revisions. Subsequently, a revised protocol was submitted in July 2019, after further revisions to the ICU procedures, and we are currently awaiting feedback/approval from the IACUC.
- The new protocol was approved on 11/12/2019

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.

- The second set of experiments is on hold due to COVID-19 pandemic.
- We plan to resume experiments as soon as institutional guidance for resuming NON-COVID-19 work allows and complete planned experiments.

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.

Nothing to Report

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals.

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).*

- Rex E. Atwood MD, Dana M. Golden, Stephen A. Kaba, and Matthew J. Bradley **(2020).** Characterization of the Cortisol Response to Traumatic Hemorrhage and Intra-Abdominal Sepsis Models in Cynomologus Macaques. Molecular and Cellular Endocrinology (**submitted**)

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

Nothing to Report

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year*

(international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

Nothing to report

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

Nothing to Report

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

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."

Name:	Dr. Anke Scultetus
Project Role:	PI
Nearest person month worked:	2
Contribution to Project:	Overall project coordination, experimental procedures/intensive care unit animal care
Name:	Dr. Matthew Bradley
Project Role:	Associate PI
Nearest person month worked:	9
Contribution to Project:	Experimental procedures/intensive care unit NHP management
Name:	Rex Atwood
Project Role:	Surgical Research Resident
Nearest person month worked:	8
Contribution to Project:	Experimental procedures/intensive care unit NHP management
Name:	John Mares
Project Role:	Research Associate
Nearest person month worked:	9
Contribution to Project:	Experimental procedures, blood draws, laboratory analysis, animal care
Name:	Crystal Leonhardt
Project Role:	Protocol Coordinator
Nearest person month worked:	No change
Contribution to Project:	Experimental procedures, blood draws, laboratory analysis, animal care
Name:	Babita Parajuli
Project Role:	Research Assistant
Nearest person month worked:	No change
Contribution to Project:	Assistance with experimental procedures, blood draws, animal care
Name:	Stephen Kaba
Project Role:	Senior Scientist
Nearest person month worked:	No Change
Contribution to Project:	Laboratory analysis
Name:	Dr. Daniel Chertow, MD
Project Role:	Critical Care Medicine
Nearest person month worked:	No Change
Contribution to Project:	Assistance with modification to sedation procedures and ICU care

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

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

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.