AWARD NUMBER: W81XWH-19-2-0040

TITLE: Anti-complement Therapy to Improve Mortality and Morbidity for Traumatic Hemorrhage during Prolonged Field Care and Prolonged Damage Control Resuscitation

PRINCIPAL INVESTIGATOR: Yansong Li

CONTRACTING ORGANIZATION: The Geneva Foundation, Tacoma, WA

REPORT DATE: October 2020

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2020	Annual	15Sep2019-14Sep2020

4. TITLE AND SUBTITLE
Anti-complement Therapy to Improve Mortality and Morbidity for Traumatic Hemorrhage during Prolonged Field Care and Prolonged Damage Control Resuscitation

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-19-2-0040

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. Yansong Li

E-Mail: yli@genevausa.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

The Geneva Foundation
917 Pacific Ave. Ste 600
Tacoma, WA 98402

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
Bleeding (hemorrhage) after trauma remains a leading cause of death. Traumatic hemorrhage (TH) causes early innate immune response and clotting disorders. An inappropriate innate immune response after TH leads to an abnormal release of inflammatory mediators that contribute to early inflammation-mediated multi-organ failure (MOF) and death. Complement cascade (ComC) as a "first line defense" and a master alarm system of the innate immunity, represents a key mechanism to prime overzealous cytokine storm and thromboinflammation that contribute to multi-system inflammatory syndrome (MSIS)-mediated MOF after TH. The purpose of this study is to develop and validate anti-ComC therapies aimed at mitigating the MSIS-induced MOF and increasing survival, thereby improving outcomes during prolonged field care and/or prehospital scenario for TH patients.

15. SUBJECT TERMS
Trauma, hemorrhage, multi-organ failure, mortality, complement cascade, multi-system inflammatory syndrome, thromboinflammation, complement inhibition, prolonged field care

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	9	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Traumatic hemorrhage (TH) is a significant global burden in civilians and military service members. Current prehospital/hospital care of the critically ill trauma patients remains primarily supportive and fails to address the destructive influence of unchecked inflammation-mediated multi-organ failure (MOF). Complement cascade (ComC) as an important part of innate immune system represents a key mechanism to prime overzealous cytokine storm and thromboinflammation that contribute to multi-system inflammatory syndrome (MSIS)-mediated MOF after TH. This DoD project is to develop and validate new anti-ComC as an effective therapy for the critically ill trauma patients to improve the poor outcomes of MSIS-induced MOF and mortality.

2. KEYWORDS:

Trauma, hemorrhage, multi-organ failure, mortality, complement cascade, multi-system inflammatory syndrome, thromboinflammation, complement inhibition, prolonged field care.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Project milestones:
1st Quarter: Animal protocol approval by the local IACUC and ACURO.
2nd-3rd Quarters: Completion of animal model development.
3rd-5th Quarters: Completion of the animal study of Coversin and C1 inhibitor efficacy in a swine TH model during a short-term PFC (10h).
4th-6th Quarters: Completion of the measurement of PK and PD profile of Coversin and C1 inhibitor, analysis of ComC, CoaC, FibC, KinC, inflammation, MOF, and endotheliopathy (10h).
7th-10th Quarters: Completion of the animal study of Coversin and C1 inhibitor efficacy in a swine TH model during a long-term PFC (24h).
7th-11th Quarters: Completion of the assessment of PK and PD profile of Coversin and C1 inhibitor, evaluation of ComC, CoaC, FibC, KinC, inflammation, MOF, and endotheliopathy (24h).
7th -12th Quarters: Completion of the dada summary and IND submission.

What was accomplished under these goals?

As lockdowns of COVID 19, we have been unable to carry on with the grant-funded large animal work at the USAISR since March 2020, which has significantly upended our research plans and research activities. To mitigate the impact, we have developed smart solutions, carefully considered optimal strategies, and applied cross-team/organization collaborations to achieve following outcomes:
1. Animal protocol approved by the local IACUC and ACURO.
2. Built/assembled a highly experienced multidisciplinary team including a PI, 2 experienced research scientists, and a research technician.
3. Established CRADAs with the Akari Therapeutics (London, UK) and the Pharming Technologies N.V. (Leiden, Netherlands).
4. Obtained the C5 inhibitor (Coversin) and C1 inhibitor via the CRADAs from the Akari Therapeutics and the Pharming Group N.V. respectively.
5. Proactively planned and scheduled swine study for the 5th quarter through cross-team collaboration.
6. Completed the test and characterization of the USAISR's new shock tube with dry runs and animal cadavers. We conclude that 1) the new expansion cone behaves as expected, with higher magnitude exposures possible by reducing the transit distance and time from the membranes for blast testing; and 2) the shock tube using helium is able to deliver a blast wave dose with consistency needed to conduct sound scientific experimentation. For details please see below Appendices.
7. Conducting data analysis and preparing 4 manuscripts.

8. Submitted a manuscript (under revision),
9. Three abstracts accepted for oral presentation in 2020 MHSRS and 2019 IFSS. For details please see below appendices.
10. Submitted 3 full proposals (1 JWMRP, 1 BAA1, and 1 PRMRP-TTDA).

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

1. The favorable efficacy and important findings of Coversin and C1 inhibitor in mitigating the MSIS-induced MOF and increasing survival in TH models have been disseminated at national (MHSRS, AAST and Shock) and international (International Complement Workshop, European on Complement in Human Disease, Intensive Care Society, International Federation of Shock Societies, NATO) conferences, DoD Website of Science & Technology Efforts & Programs (Prevention, Mitigation, and Treatment of Blast Injuries).

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

1. Carefully consider optional strategies to plan and conduct project experiment.
2. Explore new cross-team/organization collaborations and keep making progress.
3. Initiate and complete the model development.
4. Initiate the efficacy study of the short-term and complete the PK and PD experiments,
5. Train the new research technician.
6. Maintain good communication with our team, animal facility, grant/contract manager, department chief, and science officer to keeping the research going.
7. Conduct data analysis and prepare/submit manuscripts/press release/patent disclosure.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Amid ongoing problematic COVID-19 pandemic that has nixed our large animal study, we have faced many unexpected/unpredictable challenges and anticipate that are continuing to significantly impede our research activities and the project's milestones. In order to mitigate the damages, we are carefully pursuing optimal strategies and implementing following smart solutions for dealing with the problems:

1. Keep good communication with our team members and animal facility to keeping the research going.
2. Establish new cross-team collaborations and keep making progress.
3. Reach out to the grans/contract manager, department chief and science officer, and share how the crisis is affecting our work and how we plan to keep making progress.
4. Carefully prepare face-to-face interaction at the USAISR according to the USAISR's guidance.
5. Safeguard our team's mental health.
6. Cross-train staff to reduce the likelihood of institutional skill/knowledge being locked up with one person and to maximize shared research resources at the USAISR.
7. Continue to analyze data and prepare manuscripts.
8. Keep searching/reading research articles to gain new knowledge.
9. Take on webinar/online courses/conferences to build skills.
10. Prepare and write SOPs for the research project.
11. Search and purchase reagents and equipment for the research project.

Changes that had a significant impact on expenditures

The CoVID 19 crisis has upended our research activities including hiring staff and performing animal study that significantly impact on the expenditures at less cost than anticipated.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Five changes in use or care of vertebrate animals are listed as follows: 1) change of animal strain from Yorkshire swine (30-40kg) to Micro-Yucatan Miniature Swine (10-15kg) because our shock tube is not large enough for >30 kg Yorkshire swine; 2) request placement of jugular vein catheter for drug and fluid administration and replacement of femoral artery catheter with carotid artery catheter that makes an exteriorized catheter easily; 3) change of body weight range from Micro-Yucatan Miniature Swine (10-15kg) to Mini-Yucatan Miniature Swine (22-30kg) because their larger size are more suited for instrumentation; 4) request placement of DSI catheter in the femoral artery to monitor ECG; and 5) request 2 model development animals to determine conditions of blast injury and hemorrhagic shock. IACUC/ACURO approved those changes on Oct 17, 2018 and Mar 2, 2020 respectively. For details please see below Appendices.

Si

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

1. Li Y, Dubick MA, Yang Z, Barr JL, Gremmer BJ, Lucas ML, Necsoiu C, Jordan BS, Batchinsky AI, Cancio LC. Distal organ inflammation and injury after resuscitative endovascular balloon occlusion of the aorta in swine severe hemorrhagic shock (PLOS One, under revision).
2. Clinical significance of complement terminal pathway activation and C5 inhibition to improve organ failure and mortality after trauma (in preparation).
3. Early complementopathy and endotheliopathy in severe polytrauma patients (in preparation).
4. New milestones ahead in complement-targeted therapy for acute lung injury and acute respiratory distress syndrome (in preparation).

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

1. Dubick MA, Barr JL, Thomas TW, Li Y. Indices of inflammation and oxidant stress after combined blast injury and hemorrhage in rats treated with complement inhibitors (IFSS, 2019).
2. Yang Z, Pusateri AE, Hill-Pryor CD, Garcia PA, Bernetskie ST, Edsall PR, Cap AP, Walters TJ, Wade CE, Dubick MA, Cancio LC, and Li Y. Clinical significance of early complement terminal pathway activation in military casualties and prehospital inhibition of complement C5 improves survival and mitigates multi-organ damages after traumatic hemorrhage. MHSRS (oral presentation, 2020).
3. Li Y, Yang Z, Dubick MA, Garcia PA, Bernetskie ST, Edsall PR, Hill-Pryor CD, Walters TJ, Cap AP, Wade CE, Pusateri AE, and Cancio LC. Clinical significance of early HMGB release in military casualties and therapeutic targeting HMGB1 to reduce mortality and protect multi-organ injury after traumatic hemorrhage. MHSRS (oral presentation, 2020).
For details, please see below Appendices.

- **Website(s) or other Internet site(s)**

1. Joint press release entitled “Akari Therapeutics announces the signing of a Cooperative Research and Development Agreement (CRADA) with the U.S. Army Institute of Surgical Research (USAISR) combined with positive pre-clinical results and ongoing work in Battlefield Trauma” (in preparation).

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

1. Li Y, Cancio LC, Pusateri AE, Nunn Miles, Yang Z, Dubick MA, Walters TJ, and Cap AP. Coversin fo ruse to reduce mortality and protect organ injury after trauma and hemorrhagic shock (invention disclosure, in preparation).

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Yansong Li
Project role: PI
Research identifier: 60318
Nearest person month worked: 9
Contribution to project: Participated in 1) IACUC protocol writing and submission, 2) establishment of collaboration with the Akari Therapeutics Plc. (London, UK) and the Pharming Technologies B.V. (Leiden, Netherlands), 3) new shock tube characterization with dry runs and swine cadavers, 4) personnel hiring and team building, 5) project design and plan, 6) data analysis, 7) writing and submission of manuscripts, abstracts, and proposals; and 8) preparing press release and invention disclosure.
Funding support: W81XWH1920040 and CCCRP (P_010_2020)

Name: Zhangsheng Yang
Project role: Co-I
Research identifier: 48088
Nearest person month worked: 7.5
Contribution to project: Participated in 1) IACUC protocol writing and preparation, 2) new shock tube characterization with dry runs and swine cadavers, 3) personnel hiring, 4) project plan and preparation, 5) data analysis, and 6) writing and preparation of manuscripts, abstracts and proposals.
Funding support: CCCRP (P_010_2020) and W81XWH1920040

Name: Corina Necsoiu
Project role: Co-I
Research identifier:
Nearest person month worked: 2.5
Contribution to project: Participated in 1) IACUC protocol preparation, 2) personnel hiring, and 3) project design, plan and preparation.
Funding support: W81XWH1920040

Name: Tamara Fraker
Project role: Research technician
Research identifier:
Nearest person month worked: 2
Contribution to project: Completed her hiring in-process and online training, and participated in reading the research related articles and protocols, searching/ordering reagents/equipment, and revising/editing manuscripts and proposals.
Funding support: W81XWH1920040

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

Request change of PI of IACUC protocol (A18-022) from Dr. Yansong Li to Dr. Corina Necsoiu because Dr. Necsoiu has more experience in large animal ICU and Dr. Li is busy with other animal protocol. For details please see below Appendices.

What other organizations were involved as partners?

1. Established a CRADA entitled “Complement inhibition in animal models of trauma and hemorrhage” with the Akari Therapeutics Plc. (London, UK) and obtained Coversin from the Akari Therapeutics Plc.
2. Established a CRADA entitled “Efficacy of C1 inhibitor in animal models of trauma and hemorrhage” with the Pharming Technologies B.V. (Leiden, Netherlands) and obtained C1 inhibitor from the Pharming Technologies B.V.

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHARTS: Please see below Appendices.

9. APPENDICES:

1) Performance of ISR Large Shock Tube
2) Addendums (significant changes in use or care of vertebrate animals)
3) Publications (conference presentations)
4) Addendum (a change of the protocol PI)
5) Quad Charts