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TITLE: Development and Validation of a Novel Immunotherapy for Traumatic Hemorrhage

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14. ABSTRACT

Despite recent advances in hemorrhage control, traumatic hemorrhage (TH)remains a leading cause of mortality among military and civilian trauma patients. Hemorrhage after trauma is the primary cause of death on the battlefield accounting for 50%. Approximately 90% of battlefield casualties die in the prehospital environment. In addition, hemorrhage is associated with 85% of potentially survivable death in the recent conflicts. Early inflammatory response especially in complement C5 and damage associated molecular patterns represent more generalizable biological principles, which critically regulate systemic inflammatory response syndrome, compensatory anti-inflammatory syndrome, endotheliopathy, and persistent inflammation/immunosuppression and catabolism syndrome, which contribute to multi-organ failure (MOF) and mortality after TH. Therefore, early modulation of these two cascades constitutes a most effective therapeutic principle for the treatment of MOF and the improvement of survival after TH.

This project builds on our previous works and well-established capabilities. The program of trauma immunomodulation has successfully proven that anti-C5 or anti-HMGB1 therapy increases survival, improves metabolism and hemodynamics, reduces resuscitation fluid volumes, modulates systemic and local inflammatory responses, and mitigates MOF in a rat TH model. However, the efficacy of inhibition of C5 and/or HMGB1 therapeutic approaches has not been validated in a large animal trauma model at a prolonged field care (PFC) setting. Therefore, this project is to validate the effectiveness of early administration of Coversin (C5 inhibitor) and/or CX-01 (HMGB1 inhibitor) therapies aimed to attenuate morbidity and mortality after TH during PFC and prolonged damage control resuscitation.

15. SUBJECT TERMS
Immunotherapy, hemorrhage control, polytrauma, damage control resuscitation, prolonged field care, prehospital, systemic inflammatory response syndrome, multi-organ dysfunction syndrome

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	11	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

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

Traumatic hemorrhage (TH) is a major cause of death on the battlefield as well as for in the civilian world. The causes of battlefield deaths have remained unchanged over the last decade despite recent advances in treatment protocols. TH-involved ischemia/reperfusion injury, and trauma-related therapeutic approaches (blood transfusion, volume resuscitation, instrumentation, surgical damage control, extracorporeal membrane oxygenation, etc.) that play a major role in the systemic inflammatory response syndrome (SIRS), the compensatory anti-inflammatory response syndrome (CARS), endothelio[pahty (EPT), and persistent inflammation/immunosuppression and catabolism syndrome (PICS). SIRS and CARS ultimately lead to injury-related multi-organ failure (MOF). MOF represents a leading cause of late mortality following severe trauma. Current prehospital care of the critically ill civilian and military trauma patients is primarily supportive for TH patients and does not address the destructive influence of unchecked inflammation-mediated MOF. Based on these premises, the lack of effective pharmacological solutions for MOF following TH, with potential for prehospital use, is a serious unmet need, which will be particularly important for severely injured patients with TH in future prolonged field care scenarios.

A great challenge is that multiple factors/cascades after TH contribute to MOF and mortality, and simply targeting one of the regulatory factors/cascades may have limited effects. Compelling evidence suggests that complement C5 and high mobility group box 1 (HMGB1) represent more generalizable biological principles, which critically regulate SIRS, CARS, EPT, and PICS after TH. Therefore, early modulation of these two cascades constitutes the most effective therapeutic principle for the treatment of MOF and the improvement of survival after TH.

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

Traumatic hemorrhage, multi-organ failure, mortality, system inflammatory response syndrome, endotheliopathy, C5 inhibition, HMGB1 inhibition, prolonged field care.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1. Optimize the Coversin and CX-01 treatment regimen in a swine TH model	**Timeline (Months)**	**USAISR**	**Project Status**
Major Task 1. Assess PK and PD profiles of Coversin and CX-01	Months	Drs. Li, Cancio, Dubick, Walters, and Yang	Not initiated
Subtask 1. Local IACUC and ACURO approval	1-3	Drs. Li, Walters, and Yang	
Subtask 2. Determine PK profile in swine TH model	4-10	Drs. Li, Yang, and Dubick	
Subtask 2.1. Characterize PK profile of Coversin in swine TH model	4-7	Drs. Li and Yang	
Subtask 2.2. Characterize PK profile of CX-01 in swine TH model	6-10	Drs. Li and Yang	
Subtask 3. Determine PD profile in swine TH model	5-11	Drs. Li, Yang, Cancio, Dubick, Walters	
Subtask 3.1. Characterize PD profile of Coversin in swine TH model	5-8	Drs. Li and Yang	
Subtask 3.2. Characterize PD profile of CX-01 in swine TH model	7-11	Drs. Li and Yang	

Subtask 3. Determine drug-related immunotoxicity in swine TH model	4-12	Drs. Li, Dubick and Yang	
Subtask 3.1. Evaluate Coversin immunotoxicity in swine TH model	4-9	Drs. Li and Yang	
Subtask 3.2. Assess CX-01 immunotoxicity in swine TH model	6-12	Drs. Li and Yang	
Milestone(s) Achieved. 1) IACUC protocol/ ACURO approval; and 2) 3 optimal treatment regimens identified	*12*	Drs. Li, Cancio, Dubick, Walters, and Yang	
Specific Aim 2. Validate the efficacy of the 3 regimens in a swine TH model with fresh whole blood transfusion			
Major Task 2. Test the efficacy of the 3 regimens in a swine TH model with fresh whole blood transfusion		Drs. Li, Cancio, Dubick, Walters, and Yang	Not initiated
Subtask 1. Perform animal study to test the efficacy of the Coversin optimal regimen	13-24	Drs. Li and Yang	
Subtask 2. Perform animal study to test the efficacy of the CX-01 optimal regimen	13-24	Drs. Li and Yang	
Subtask 3. Perform animal study to test the efficacy of the Coversin+CX-01 optimal regimen	13-24	Drs. Li and Yang	
Milestone(s) Achieved: 1) 1 lead treatment regimen identified; and 2) Submission of full IND application	24	Drs. Li, Cancio, Dubick, Walters, and Yang	
Specific Aim 3. Validate the efficacy of the 3 regimens in a swine TH model with Hex resuscitation			
Major Task 3. Evaluate the efficacy of the 3 regimens in a swine TH model with Hex resuscitation		Drs. Li, Cancio, Dubick, Walters, and Yang	Not initiated
Subtask 1. Conduct animal study to test the efficacy of the Coversin optimal regimen	25-36	Drs. Li and Yang	
Subtask 2. Conduct animal study to test the efficacy of the CX-01 optimal regimen	25-36	Drs. Li and Yang	
Subtask 3. Conduct animal study to test the efficacy of the Coversin+CX-01 optimal regimen	25-36	Drs. Li and Yang	
Milestone(s) Achieved: 1) 1 lead treatment regimen identified; and 2) Submission of full IND application	36	Drs. Li, Cancio, Dubick, Walters, and Yang	

What was accomplished under these goals?

Objective: To optimize the Coversin and CX-01 treatment regimen in a swine TH model.

Major activities: 1) Research team formation; 2) Conference calls with the Akari Therapeutics (London, UK) and the Chimerix Inc. (Durham,NC); 3) Alternative performance site search; 4) Proposal preparation; and 5) Participation in Webinar meeting/training..

Significant results/key outcomes: We (USAISR) have established CRADAs with the Akari Therapeutics and the Chimerix Inc. to evaluate the efficacy of Coversin (C5 inhibitor) and CX-01 (HMGB1 inhibitor). We have built/assembled a highly experienced multidisciplinary team including Dr. Yansong Li (PI), COL (ret.)/Dr. Leopoldo Cancio (Co-I), Dr. Milomir Simovic (Co-I) and Ms. Tamara Fraker (Research Technician). We have submitted a proposal entitled “Development of a novel NLRP3 inflammasome-targeted therapeutic to treat traumatic hemorrhage-induced multiple-organ failure during prolonged field care” (PRMRP-IIRA). We have

attended International Complement Society 2021 Webinar Symposium: Complement and Inflammation and Emulate's organ-on-a-chip Webinar training

Major challenges: Due to research capacity limitations at the USAISR caused by COVID-19 pandemic, we have not initiated the preclinical experiment yet. To overcome the USAISR's capacity limitations, we have routinely communicated/discussed the challenges/plans with our the Co-Is, team members, the USAISR's leadership, the Geneva Foundation, the Science Officer/Grants Management Specialist, and the pharmaceutical companies. Meanwhile, we have proactively searched alternative performance sites and identified potential performance sites including UT Health Science Center at San Antonio, Naval Medical Research Unit San Antonio, and 59th Medical Wing Operational Medicine MDW.

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

By using our existing IACUC protocol entitled "Development and characterization of a swine model of blast injury and hemorrhage", we have trained 2 Scientist and 2 Technicians with regard to animal ICU, anesthesia, animal handling, surgery, blood sample collection/processing and analysis, blast injury, hemorrhagic shock, vital sign monitoring and recording, data entry and organization, necropsy, functional complement assays, and reagent/supply purchase.

How were the results disseminated to communities of interest?

The generated results and important findings in this project have been disseminated and shared with the civilian/military trauma research communities in the forms of oral/poster presentation in 2021 MHSRS (Kissimmee, FL), 2021 Military City USA Trauma Collaborative Research Conference (San Antonio TX), and a joint press release (New York and London, March 04, 2021 Globe Newswire, https://www.globenewswire.com/en/news-release/2021/03/04/2187148/0/en/Akari-Therapeutics-Announces-a-Cooperative-Research-and-Development-Agreement-CRADA-with-the-U-S-Army-Institute-of-Surgical-Research-USAISR-for-Nomacopan-in-Trauma.html).

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

Due to the USAISR's limited research capacity caused by the COVID-19 pandemic, the USAISR is no longer able to support this project. To achieve the project milestones/goals, we are planning to perform this project in the Division of Trauma Research at the UT Health Science Center at San Antonio (UTHSCSA). The UTHSCSA has distinguished animal facilities and animal care system, clinical trauma research experts, laboratory support, and a world-class research core facilities/services as well as an excellent research environment, where is best suited for our research program applying a translational **BBB** approach (**B**edside to **B**ench and **B**ack). Our detailed plans are as follows:

1. We have assembled a new exceptional multidisciplinary team consisting of team members: Dr. Li (PI, a senior trauma immunologist), and Co-Is: COL (ret.)/Dr. Cancio (a physician-scientist with his expertise at critical care), Dr. Susannah Nicholson (a physician-scientist with expertise at trauma-induced multi-organ failure), Dr. Batchinsky (a senior research scientist with his expertise at swine models of trauma-induced multi-organ failure), and Dr. Simovic (a senior trauma physiologist).
2. We have submitted the CRADAs between the pharmaceutical companies (Akari and Chimerix) and the Geneva Foundation.
3. We are preparing a package of documents for changing performance site and are going to submit it to USAMRDC for an approval.
4. We are preparing a new IACUC protocol and doing online/in-person trainings of animal research, ethics, and compliance.

5. We are proactively setting up the new animal surgical room and wet laboratory at the UTHSCSA.
6. We are planning to hire a new postdoctor and a new technician.
7. We are exploring new cross-team/organization collaborations at the UTHSCSA.
8. We are planning to initiate the animal study on December 2021.
9. We are preparing/writing new SOPs for our animal/laboratory experiments.
10. We are preparing/writing two manuscripts.

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 continuous problematic COVID-19 pandemic and limited research capacity at the USAISR that have significantly upended our research plans and nixed research activities. To keep this project on track, we have developed strategic plans as follows:

1. We are planning to change the performance site from the USAISR to the UTHSCSA (see above).
2. We are going to keep a good communication with our team members, the Geneva Foundation, the Science Officer/Grants Management Specialist, and the collaborator at the UTHSCSA and the pharmaceutical companies.

3. We are proactively visiting/setting experimental laboratories at the UTHSCSA, searching equipment, doing online/in person trainings, and preparing IACUC protocols.
4. We are going to cross-train the staff to reduce the likelihood of institutional skill/knowledge being locked up with one person and to maximize shared research resources at UTHSCSA.
5. We are going to searching/reading research articles to gain new knowledge.
6. We will take on webinar/online courses/conferences to gain insights and build skills.
7. We are going to prepare and write SOPs for this research project.

Changes that had a significant impact on expenditures

The COVID-19 crisis and USAISR's limited capacity have upended our research activities including performing animal study and personnel hiring 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

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

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

Journal publications.

1. Li Y, 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. 2020 Nov 17; 15(11):e0242450). *Acknowledgement of federal support (yes).*
2. Bermudez T, Sammani S, Song JH, Quijada H, Valera DG, Hernon VR, Kempf CL, Garcia AN, Burt J, Camp SM, Hufford M, Cress AE, Desai AA, Natarajan V, Jacobson JR, Dudek SM, Cancio LC, Alvarez J, Rafikov R, **Li Y**, Zhang DD, Casanova N, Bime C, Garcia JGN. An eNAMPT-neutralizing mAb reduces rat and porcine lung injury severity in preclinical ARDS/VILI models (JEM, submitted). *Acknowledgement of federal support (yes).*

3. Yang Z, Le TD, Simovic MO, Liu B, Fraker TL, Cap AP, Wade CE, DalleLucca JJ, Li Y. Intercommunication of tri-opathies: Complementopahty, endotheliopathy and coagulopathy, and their impacts on clinical outcomes in severe poly-trauma patients (Front Immunol, submitted). *Acknowledgement of federal support (yes).*
4. Yang Z, Nunn MA, Le TD, Simovic MO, Edsall PR, Liu B, Barr JL, Lund BJ, Hill-Pryor CD, Pusateri AE, Cancio LC, Li Y. Terminal complement activation in military casualties and the potential of complement C5 inhibition to improve organ function and survival in trauma. (Sci Transl Med, submitted). *Acknowledgement of federal support (yes).*
5. Yang Z, Simovic MO, Edsall PR, Liu B, Tomas S Cancio, Brian J. Lund, Andriy I. Batchinsky Cancio LC, Li Y. Systemic HMGB1 Release in Combat Casualties and HMGB1 Inhibition to Improve Organ Function and Survival (in preparation). *Acknowledgement of federal support (yes).*

For details, please see below Appendices (Attachment #1).

Books or other non-periodical, one-time publications

Nothing to Report

Other publications, conference papers and presentations.

1. Yang Z, Cap AP, Simovic MO, Cancio LC, Wade CE, Li Y. Intercommunication of tri-opathies: Complementopathy, endotheliopathy and coagulopathy, and their impacts on clinical outcomes in severe polytrauma patients [oral presentation, 2021 MHSRS (Kissimmee, FL) and 2021 Military City USA Trauma Collaborative Research Conference (San Antonio TX)].
2. Tamara L. Fraker, MS, Zhangsheng Yang, PhD, Bryan S. Jordan, RN, Michael L. Lucas, MS, Tomas S. Cancio, BS, Milomir O. Simovic, PhD, MD, Corina Necsoiu, MD, Thomas J. Walters, PhD, COL Andrew P. Cap, MD, PhD, Yansong Li, MD, and COL (Ret.) Leopoldo C. Cancio, MD. Development of a porcine model of blast injury and hemorrhagic shock: systemic activation of complement pathways, HMGB1 release and coagulation profile [poster presentation, 2021 MHSRS 2021 MHSRS (Kissimmee, FL) and 2021 Military City USA Trauma Collaborative Research Conference (San Antonio TX)].
3. Corina Necsoiu, MD, Zhangsheng Yang, PhD, Tamara L. Fraker, MS, Bryan S. Jordan, RN, Michael L. Lucas, MS, Tomas S. Cancio, BS, Milomir O. Simovic, PhD, MD, Thomas J. Walters, PhD, COL Andrew P. Cap, MD, PhD, COL (Ret.) Leopoldo C. Cancio, MD, and Yansong Li, MD. Indices of organ damage in a swine model of blast injury and hemorrhage [poster presentation, 2021 MHSRS 2021 MHSRS (Kissimmee, FL) and 2021 Military City USA Trauma Collaborative Research Conference (San Antonio TX)].

For details, please see below Appendices (Attachment #2).

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

1. Akari Therapeutics announces a Cooperative Research and Development Agreement (CRADA) with the U.S. Army Institute of Surgical Research (USAISR) for Nomacopan (Coversin) in Trauma: Study of porcine model of blast injury and haemorrhagic shock underway with USAISR as part of the development of a clinical path for the use of Nomacopan (Coversin) to treat trauma (Joint press release, New York and London, March 04, 2021 Globe Newswire, https://www.globenewswire.com/en/news-release/2021/03/04/2187148/0/en/Akari-Therapeutics-Announces-a-Cooperative-Research-and-Development-Agreement-CRADA-with-the-U-S-Army-Institute-of-Surgical-Research-USAISR-for-Nomacopan-in-Trauma.html.

For details, please see below Appendices (Attachment #3).

- **Technologies or techniques**

Nothing to Report

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

Li Y, Cancio LC, Pusateri AE, Nunn Miles, and Yang Z. Coversin for the use 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: 6
Contribution to project: Participated in 1) IACUC protocol preparation, 2) an establishment of collaboration with the Akari Therapeutics Plc. and the Chimerix Inc. for providing Coversin and CX-01 respectively, 3) personnel hiring/training and team building, 4) project design and strategic plan, 5) searching/identifying the alternative performance sites, 6) writing and submitting manuscripts, abstracts, and proposals; and 7) preparing/submitting press release and invention disclosure.
Funding support: W81XWH1920040

Name: Leopoldo Cancio
Project role: Co-I
Research identifier:
Nearest person month worked: 1
Contribution to project: Participated in 1) IACUC protocol preparation, 2) personnel hiring/training and team building, 3) project design and strategic plan, 4) searching/identifying alternative performance sites, 5) writing and submitting manuscripts, abstracts, and proposals; and 6) preparing/submitting press release and invention disclosure.
Funding support: Dr. Cancio’s salary is paid by the federal government.

Name: Milomir Simovic
Project role: Co-I
Research identifier:
Nearest person month worked: 4

Contribution to project: Participated in 1) IACUC protocol preparation, 2) attending online/in-person trainings, 3) data analysis/summary, and 4) writing and submitting manuscripts, abstracts, and proposals.
Funding support: W81XWH1920040

Name: Tamara Fraker
Project role: Research Technician
Research identifier:
Nearest person month worked: 6
Contribution to project: Participated in 1) searching/ordering reagents/devices, 2) attending online/in-person trainings, 3) data analysis/summary, 4) revising/editing manuscripts and proposals, and 5) preparing/submitting abstract/poster.
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?

Nothing to Report

What other organizations were involved as partners?

1. We had monthly phone conference with the Akari Therapeutics Plc. to discuss our study progress and experimental plans, and gain insightful comments.
2. We routinely shared and discuss our experimental design, status and plans with the Chimerix Inc..

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHARTS: Attached

9. APPENDICES:

1) Attachment #1: Publications
2) Attachment #2: Conference presentation
3) Attachment #3: Joint press release