AWARD NUMBER: W81XWH-19-2-0061

TITLE: Identification of Mechanisms Underlying the Effects of Plasma Inclusive Resuscitation in Major Thermal Injury on Hemostasis and Vascular Homeostasis

PRINCIPAL INVESTIGATOR: Jeffrey W. Shupp, MD

CONTRACTING ORGANIZATION: MedStar Health Research Institute
Washington, DC

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	30SEP2019-29SEP2020

4. TITLE AND SUBTITLE

Identification of Mechanisms Underlying the Effects of Plasma Inclusive Resuscitation in Major Thermal Injury on Hemostasis and Vascular Homeostasis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-19-2-0061

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Melissa McLawhorn RN BSN, Lauren Moffatt PhD, Dr. Jeffrey Shupp, MD

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

MedStar Health Research Institute
108 Irving Street, NW
George Hyman Research Bldg. Rm. 304
Washington, DC 20010

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

Recently, investigation is re-emerging on optimizing burn shock resuscitation. Areas of interest include a variety of strategies for reducing volume requirements (as would be needed in an austere environment—or where prolonged field care is required). Colloid is again being trialed in attempts to improve the response to vascular leakage, intravascular volume expansion and reduction of volume. Off-the-shelf colloid options include human serum albumin and fresh frozen plasma (FFP). Many prescribers of FFP report great response in regards to intravascular volume expansion with minimal side effects, though some reports exist of transfusion associated complications. Unfortunately, little is known about the impacts of these transfusion products on thermally-injured patients. Hence this proposal outlines a study that will elucidate the impact of a plasma-inclusive resuscitation (PIR) which will advance strategies for prolonged field care (i.e. freeze-dried plasma). We hypothesize that a PIR alters coagulation homeostasis in a positive manner while combating the effects vascular endothelial dysfunction and modulating the inflammatory host response. This prospective, systematic, and comprehensive characterization of FFP *and* its impacts on hemostatic and inflammatory potential of burn patients will be broadly applicable because it will serve to advance the body of knowledge available on blood products. By addressing these and previously cited gaps in research, the refinement of both plasma products and transfusion strategies for a large population of patients may be accelerated.

15. SUBJECT TERMS
FFP, resuscitation, inflammation, endotheliopathy

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

Accomplishments:

What were the major goals of the project?

Major Task 1: Secure Regulatory Approval and Plan for Screening and Enrollment Coverage: **100%**

Subtask 1: Clinical study/human subjects protocol preparation; data collection systems setup **100%**

Subtask 2: Material and supply acquisition and on-call scheduling for screening; Staff training for enrollment, sample acquisition, and data abstraction **100%**

Local IRB Approval and submission to HRPO: **100%**
Milestone Achieved: HRPO Approval

Major Task 2: Patient Enrollment and Sampling: **0%**

Subtask 1: Enroll patients and obtain complete sample sets as described **0%**

Subtask 2: Obtain clinical data and enter into database in real time **0%**

Subtask 3: Transfer samples to collaborating sites for assay **0%**

Subtask 4: Establish multi-donor pool of healthy human plasma **0%**

Milestone(s) Achieved: samples obtained and transferred for assay, clinical data set being compiled **0%**

Specific Aim 1: To assess changes in the inflammatory state of burn patients undergoing a resuscitation protocol that includes plasma transfusion

Major Task 3: Measure inflammatory markers: **0%**

Subtask 1: RNA isolation and whole genome microarray analysis (PAXgene blood) **0%**

Subtask 2 (Aim 1a. Understand the inflammatory potential of plasma products): Inflammatory marker protein panel quantification including IL-1b, IL-6, IL-10, IL-12p70, TNF-α, TNF-α R1/R2 and sIL-6 receptor (blood samples and FFP satellites/samples) **0%**

Milestone(s) Achieved (Aim 1b. Identify and characterize a further modulation of the host response by FFP administration in the setting of major thermal trauma): Inflammatory data obtained and integration with clinical and other data **0%**

Specific Aim 2: To assess changes in the vascular homeostasis and endothelial function of burn patients undergoing a resuscitation protocol that includes plasma transfusion

Major Task 4: Measure markers of endothelial integrity and assess plasma composition for extracellular vesicles: **0%**

Subtask 1 (Aim 2b. To determine the hemostatic potency of EVs present in the transfused FFP units and relate this potency to alterations in the parameters assessing endotheliopathy in each patient): Extracellular vesicle measurements in blood samples and FFP satellites/samples **0%**

Subtask 2 (Aim 2a. To evaluate the level of "endotheliopathy" (endothelial dysfunction) at baseline and after plasma transfusion): Marker protein level quantification including CD-138, sTM, neutrophil elastase-antitrypsin complex, sP-selectin (blood samples and FFP satellites/samples) **0%**

Subtask 3 (Aim 2a): Vascular leakage quantification including fibrinogen, α2-macroglobulin, VEGF, and ceruloplasmin (Saliva) **0%**

Milestone(s) Achieved: Endothelial function data obtained and integration with clinical and other data **0%**

Specific Aim 3: To assess the trajectory of clot stability in burn patients undergoing a resuscitation protocol that includes plasma transfusion

Major Task 5 (3a. To evaluate the relationship between thrombin generation intensity (procoagulant potential) and clot stability in the burn patient receiving plasma resuscitation. 3b. To determine whether observed changes in clot stability are solely derived from the transfused product): Measure markers and assessments of coagulation **0%**

Subtask 1: Mine transcriptome data for related differential gene expression (see protein markers) **0%**

Subtask 2: Marker protein level quantification and functional assays including α-TAT, fibrin monomer, D-Dimer, PAI-1, factors II, V, VII, VIII, IX, X, AT, TFPI, PC, XI, XII, plasminogen, FXIII, TAFI, C1 esterase inhibitor, etc. (blood samples and FFP) **0%**

Subtask 3: Data analysis and integration **0%**

Milestone(s) Achieved: Coagulation data obtained and integration with clinical and other data **0%**

Major Task 6: Data analysis and integration using systems biology approaches **0%**

Subtask 1: Analyze data and integrate assay results, clinical data and outcomes, functional assay results, and relationships between FFP and patient samples **0%**

Subtask 2: Produce reports and manuscripts **0%**

What was accomplished under these goals?

Major Activities:

- HRPO regulatory approval obtained
- Participant screening initiated.

Objectives:

- Obtain regulatory approval
- Train clinical research and laboratory staff for subject enrollment and sample processing
- Enroll subjects and obtain complete sample sets

Key Results/Outcomes/Findings:

- Request for re-review in accordance to HRPO concerns was reviewed by the IRB July 7, 2020 and study was determined to be less than minimal risk. Protocol v1.3 was subsequently submitted to the IRB in conjunction with the continuing review and approved Aug 24, 2020.
- A kick off training meeting was held July 29, 2020 and screening was initiated Aug 10, 2020
- Total of 92 subjects were screened during Y01Q04 with first subject enrollment occurring early in Y02Q01.

Describe the Regulatory Protocol and Activity Status (if applicable).

(a) ***Human Use Regulatory Protocols***

TOTAL PROTOCOLS: *State the total number of human use protocols required to complete this project (e.g., 5 human subject research protocols will be required to complete the Statement of Work."). If not applicable, write "No human subjects research will be performed to complete the Statement of Work."*

PROTOCOL(S): *List the identifier and title for all human use protocols needed to complete the project. Include information about the approved target number for clinical significance, type of submission, type of approval with associated dates, and performance status.*

The following format shall be used:

TOTAL PROTOCOLS: 1 human subject research protocol required to complete the Statement of Work

PROTOCOL (1 of 1 total):

Protocol [HRPO Assigned Number]: E01111.1a

Title: Identification of Mechanisms Underlying the Effects of Plasma Inclusive Resuscitation in Major Thermal Injury on Hemostasis and Vascular Homeostasis

Target required for clinical significance: 30

Target approved for clinical significance: 50

SUBMITTED TO AND APPROVED BY:

- **MedStar Health Research Institute IRB**

STATUS:

(i) Number of subjects recruited/original planned target: 0/50
Number of subjects screened/original planned target: 92/n/a
Number of patients enrolled/original planned target:0/50
Number of patients completed/original planned target: 0/30
Note: total number expected to be accrued is 30 for analysis but it is anticipated that 50 subjects may need to be enrolled to account for withdrawals, refusals to consent, screen fails.

(ii) Report amendments submitted to the IRB and USAMRMC HRPO for review:

- HRPO approval obtained July 20, 2020
- HRPO acknowledged protocol 1.3 and CR documents Sept 10, 2020

Local IRB submissions:

- Initial submission was approved Sept 9, 2019
- An amendment updating the protocol to version 1.2 and ICF was approved December 16, 2019
- Request for re-review submitted June 18, 2020 and study status updated July 7, 2020
- Annual review and an amendment updating the protocol to version 1.3 and ICF updates was approved Aug 24, 2020

(iii) Adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation: n/a

(b) Use of Human Cadavers for Research Development Test & Evaluation (RDT&E), Education or Training

TOTAL ACTIVITIES: ***No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work (SOW).***

(c) Animal Use Regulatory Protocols

TOTAL PROTOCOL(S): ***No animal use research will be performed to complete the Statement of Work***

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

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

- Continue active screening and enrollment of study participants
- Continue real time sample collection and processing
- Continue database entry of clinical data

Products: List any products resulting from the project during the reporting period. If there are no products to report for the current quarter, state "Nothing to report."

Examples of products include:

- *publications, conference papers, and presentations;*
- *website(s) or other Internet site(s);*
- *technologies or techniques;*
- *inventions, patent applications, and/or licenses; and*
- *other products, such as data or databases, biospecimen collections, germplasm, audio or video products, software, models, educational aids or curricula, instruments or equipment, data and research material, clinical or educational interventions, or new business creation.*

- Nothing to Report

Participants & Other Collaborating Organizations

What individuals have worked on the project?
Provide the following information for: (1) Project Directors (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).

Provide the name and identify the role the person played in the project. Indicate the nearest whole person month (Calendar, Academic, Summer) that the individual worked on the project. Show the most senior role in which the person worked on the project for any significant length of time. For example, if an undergraduate student graduated, entered graduate school, and continued to work on the project, show that person as a graduate student, preferably explaining the change in involvement.

Describe how this person contributed to the project. If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name: *Melissa McLawhorn*
Project Role: *Project Manager*
Nearest person month worked: *3*
Contribution to Project: *Ms. McLawhorn has developed the study protocol, informed consent, and manual of operations in conjunction with Drs. Shupp and Moffatt. She has interfaced with the local IRB to obtain regulatory approval and prepared the submission for HRPO review. Revisions of the protocol were required to secure HRPO submission there were completed and HRPO submission was send Dec 19, 2019. She has also attended a workshop on the freezer management software and started developing the sample labeling and storage guidelines.*
She has also created the on-call scheduling process and developed the on-call schedule for when the study receives HRPO approval to begin enrollment. She has started ordering necessary supplies for sample collection and processing. She has started work on developing the central RedCAP database which will serve as the primary electronic data capture system and completion guidelines for this research project and is developing education tools for the clinical staff that may need to assist with this project. She is also developing a limited set of paper case report forms for real time source data capture and is meeting with the freezer management software consultant to optimize sample labeling and storage. Ms. McLawhorn is now working with representatives from the assay sites to obtain their local IRB approval for HRPO.
Ms. McLawhorn has coordinated with representatives at HRPO to identify irregularities with local IRB determination. In conjunction with PI Dr. Jeffrey Shupp she was prepared a request to re-review the study and re-assess the risk determination. She has finalized the RedCap database buildout and labeling and freezer storage buildout. In conjunction with Drs. Moffatt and Orfeo she has finalized the Manual of Operations.
Q4: Ms. McLawhorn worked with the local IRB officials, HRPO representatives, Dr. Moffatt and PI Dr. Shupp to secure regulatory approvals. She led a kick off training meeting in late July. In conjunction with the Clinical Research Coordinator staff she has put together data entry guidelines, screening logs, and subject enrollment folders. She is assisting the Clinical Research Coordinators with day to day screening for potential subjects.

Name: *Lauren Moffatt, PhD*
Project Role: *Lab and Admin Manager*
Nearest person month worked: *1*
Contribution to Project: *Dr. Moffatt has worked with Ms. McLawhorn and Dr. Shupp on scientific aspects of the protocol and manual of operations. She has revised the grant documents as requested in order to facilitate contracting. She is working with grant collaborators to discuss supply needs for sample processing. Dr. Moffatt has been troubleshooting the planned on site platelet aggregometry and saliva assays.*
Dr. Moffatt has worked with Ms. McLawhorn to ensure the Manual of Operations is accurate and inclusive of best practice.

Changes/Problems:

a. Actual Problems or delays and actions to resolve them

Provide a description of current problems or issues that may impede performance or progress of this project along with proposed corrective action. Also 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.

For an award that includes the recruitment of human subjects for clinical research or a clinical trial, discuss any problems or barriers encountered, if applicable, and what has been done to mitigate those issues. Discussion may highlight enrollment problems, retention problems, and actions taken to increase enrollment and/or improve retention.

Nothing new to report

b. Anticipated Problems/Issues

Provide a description of anticipated problems or issues that have a potential to impede performance or progress. Also provide course of actions planned to mitigate problems or to take should the problem materialize.

Nothing to Report

Special Reporting Requirements:

Quad Charts: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.