AWARD NUMBER: W81XWH-17-2-0054

TITLE: Dried Plasma to Improve Outcomes in Polytrauma, Hemorrhage, and Trauma-Associated Sepsis (TAS): Novel Solutions for the Prolonged Field Care Environment

PRINCIPAL INVESTIGATOR: Dr. Rosemary Kozar

CONTRACTING ORGANIZATION: University of Maryland
Baltimore, MD 21201

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

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Dried Plasma to Improve Outcomes in Polytrauma, Hemorrhage, and Trauma-Associated Sepsis (TAS): Novel Solutions for the Prolonged Field Care Environment

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-2-0054

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. Rosemary Kozar
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

UNIVERSITY OF MARYLAND,
Baltimore
220 ARCH ST RM 02148
BALTIMORE MD 21201-1531

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

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Hemorrhagic shock (HS) remains the leading cause of early death among the severely injured in both the civilian and military settings, and patients that survive HS are prone to sepsis**.** As the treatment of trauma-associated sepsis (TAS) on the battlefield will be unique to prolonged field care, new therapeutic strategies that are feasible and readily translatable are urgently needed. We are proposing the novel use plasma as a primary resuscitative fluid for TAS as we anticipate that the endothelial protective effects seen after HS will also be present after TAS. However, there are logistical challenges and safety issues with the use of fresh frozen plasma (FFP) in the battlefield. We therefore will study the use of pathogen-reduced freeze dried plasma (FDP) and hypothesize that FDP- based resuscitation after TAS will be equivalent to FFP, superior to hextend, and will reduce the endotheliopathy of sepsis (EOS), mitigate vascular and end organ injury, and decrease mortality, in clinically relevant models of TAS. This hypothesis will be tested first in a rodent model to examine systemic, vascular, organ-specific pathophysiology and survival in a mouse model of HS and prolonged hypotensive resuscitation with TAS and then confirmed and expanded using a swine model of TAS to determine the modulatory effects of SDP compared to hextend on hemodynamics, end-organ function, coagulopathy and survival in a swine model of TAS.

15. SUBJECT TERMS
Hemorrhagic shock, trauma, trauma-associated sepsis, sepsis, prolonged field care, endotheliopathy of trauma, hextend, fresh frozen plasma, freeze dried plasma

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	14	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.*

The goal of the current project is to determine if plasma is an ideal fluid for resuscitation in the prolonged field care environment for trauma/hemorrhagic shock and trauma-associated sepsis (TAS). Additionally, use of a freeze dried(FD) plasma product and compared to fresh frozen plasma. We hypothesize that FD plasma- based resuscitation after TAS will be equivalent to FFP, superior to hextend, and will reduce the endotheliopathy of sepsis (EOS), mitigate vascular and end organ injury, and decrease mortality, in clinically relevant mice and swine models of TAS.

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

Hemorrhagic shock, trauma, trauma-associated sepsis, sepsis, prolonged field care, endotheliopathy of trauma, hextend, fresh frozen plasma, freeze dried plasma

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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.

	Timeline (Months)	
Specific Aim 1: To determine the effects of spray-dried plasma compared to fresh frozen plasma and hextend on systemic, vascular, organ-specific pathophysiology and survival in a rodent model of hemorrhage shock and prolonged hypotensive resuscitation with trauma associated sepsis		
Major Task 1: Obtain approval for mice experiments		Completed
Subtask 1: Obtain local IACUC approval for mouse studies. (Estimated total number of animals: 245)	0-2 Kozar	6-27-2017
Subtask 2: Obtain ARUCO approval for mouse studies	0-4 Kozar	9-18-2017
Milestone .IACUC/ARUCO approvals	4	
Major Task 2: Preparation and testing of cecal slurry		
Subtask 1: Harvest cecal slurry (25 mice)	4-5 Kozar	11-8-2017
Subtask 2: : Perform LD100 experiments	5-7 Kozar	11-28-2017
Milestone: Complete cecal slurry preparation and testing	7	
Major Task 3: Conduct short term study of HS and prolonged hypotensive resuscitation (PHR)		
Subtask 1: Perform short term hemorrhagic shock and PHR ; harvest lung tissue and collect blood. The 4 groups include: sham, HS+FDP, HS+FFP, HS+hextend	7-12 Kozar	8-1-2018
Subtask 2: Analyze lung tissue for injury, inflammation, and permeability	10-15 Kozar	In progress 50%
Subtask 3: Analyze tissue for junctional integrity	10-15 Pati	In progress 10%
Subtask 4: Analyze blood and BAL for cytokines	10-15 Pati	In progress 10%
Milestone *Complete short term mouse surgeries and analysis for HS and PHR*	15	

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.

Major Task 1: Completed with appropriate approvals

Major Task 2: A cecal slurry was made from the cecal content of 25 mice. To ascertain viability, LD 100 experiments were obtained. To do this, we first injected 400 ul of slurry into a set of three mice, two died in 24 hours but the third survived to one week at which time it was euthanized. Next, 500 ul of slurry was injected intraperitoneal into three mice and all three died in less than 24 hours. Therefore, 500 ul of slurry = LD 100

Major Task 3: We have completed the animals for prolonged hypotensive resuscitation (PHR) which entailed a trauma laparotomy then hemorrhagic shock (HS) x 90 minutes followed by prolonged hypotensive resuscitation for six hours with resuscitative fluids given to maintain MAP=60. Fluids used were hextend (HEX), fresh frozen plasma (FFP), or lyophilized plasma (LP). Two sets of animals were done, one for permeability assay using Evans blue and the second set was used to obtain blood, bronchoalveolar fluid, and lung tissue for subsequent assays.

Animals for tissue harvesting for subsequent analysis

SHAM 8
HS+PHR+HEX 8
HS+PHR+FFP 8
HS+PHR+LP 8

Animals for lung permeability (see figure 1 below)

SHAM 8
HS+PHR+HEX 8
HS+PHR+FFP 8
HS+PHR+LP 24 (8 in Fig1 plus an additional 16 animals in Fig2)

Amount of blood withdrawn to induce hemorrhagic shock:

HS+PHR+HEX 0.72 +/- 0.04 ml
HS+PHR+FFP 0.71 +/- 0.04 ml
HS+PHR+LP: 0.84 +/- 0.05 ml

Amount of fluid/blood given back during the 6 hours of PHR to maintain MAP 60 mm Hg

HS+PHR+HEX 0.40 +/- 0.04 ml
HS+PHR+FFP 0.44 +/- 0.05 ml
HS+PHR+LP 0.11 +/- 0.02 ml (Fig 1 animals)

RESULTS of Lung permeability as measured by Evan's Blue are shown in Fig.1

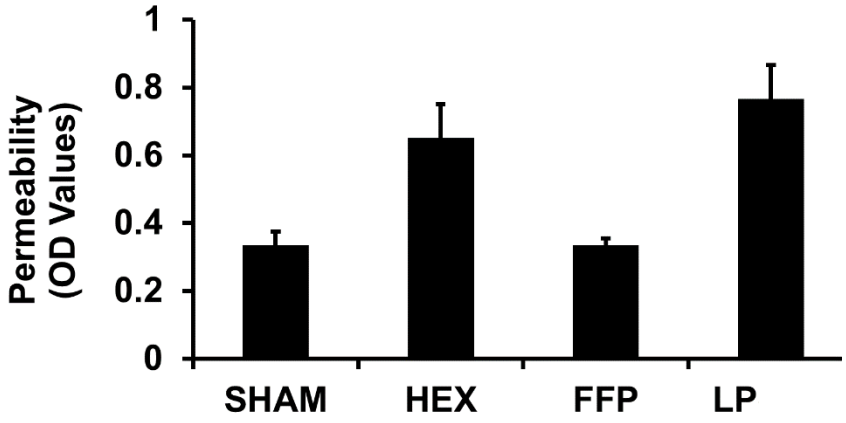


Fig 1. Lung permeability after hemorrhagic shock and prolonged hypotensive resuscitation. *There was a significant increase in permeability after HS and resuscitation with hextend and lypophilized plasma which was decreased back to sham levels with FFP.*

As we have extensive experience in our hemorrhagic shock model and have used different lyophilized products, the results of the LP group were unexpected and caused concern. We first checked basic coagulation studies of the reconstituted product and PT/PTT and fibrinogen levels were normal. When we looked at the data above showing the amount of fluid needed to maintain MAP=60 mm Hg, it appeared that the LP group received less fluid. In case hypovolemia was a cause, we resuscitated another set of animals using the same approximate volume as the FFP group received (about 0.4 mls) (Fig 2). There was still no improvement in permeability. The last thing we tried was using the volume of blood equal to 1x shed blood as this is the model that we have used extensively in the past with both FFP and LP. As shown in Fig 2, even using 0.8 mls of LP did not improve permeability.

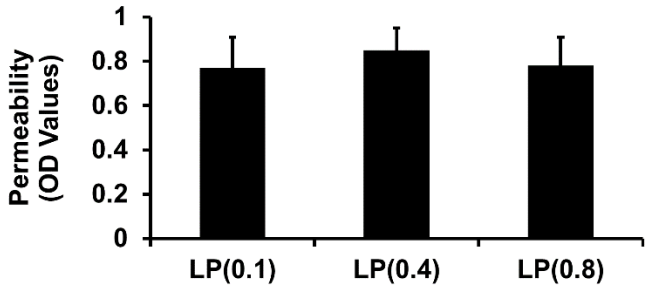


Figure 2. Lung permeability using different volumes of lyophilized plasma for resuscitation.

The next thing we investigated was the pH. We tested FFP and pH= 7.0 whereas the pH of the LP was 8.5. We believe this to be the problem. The French product is not pH balanced whereas informal conversations with other companies working on lyophilized products revealed that they pH balance with glycine.

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.

Nothing to report

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

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

We will be working on all the proposed lung tissue assays as well as blood and BAL analysis. BAL and blood was sent to Dr. Pati's laboratory in July and cut tissue samples in September. We are also working on a number of tissue assays (histology and myeloperoxidase) on the cut sections. Following completion, we will progress onto studies of TAS.

4. **IMPACT:** D*escribe 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

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 PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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:*

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.

We have encountered a number of problems.

1. We initially obtained FFP from Bonfils Blood center. After several animals not surviving the period of hemorrhagic shock and resuscitation with FFP, we contacted the company and they mistakenly sent us two units from female donors. They were replaced with male donors but the mice did not react as expected (we have used a mouse model of HS for years and have very consistent results). We subsequently have purchased additional units of blood from another blood center and they are functioning well.
2. There was a logistical issue in obtaining the FDP. It was shipped in liquid form from France but got caught up in customs in the US. By the time it arrived, the dry ice had melted and the plasma was defrosted. They subsequently sent us freeze

dried product and we constituted it. However, as shown in Fig 1 and 2, the FDP is not performing as expected which we believe is due to an issue with pH. Whether this same issue would happen in our larger swine model is not known. We have discussed this problem with Mr Malloy and are in the process of reaching out to another company (Vascular Solutions, now Teleflex) to see if they would be willing to supply us with their product.

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.

We have had to use additional mice with the issues discussed above with the FFP and then the FDP but this as of yet have not been major changes in expenditure.

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

Not applicable

Significant changes in use or care of vertebrate animals

No significant changes
local IACUC approval for mouse studies 6-27-2017
ARUCO approval for mouse studies 9-18-2017

Significant changes in use of biohazards and/or select agents

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).*

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.*

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. Describe the technologies or techniques were 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. 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;*
- *physical 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

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".

Rosemary Kozar
PI
2.1 calendar months
Completed IACUC/ARUCO/HRPO, assisted with planning, methods and analysis of data

Feng Wu
Research Associate
6.4 calendar months
Assisted with IACUC protocols, performed animal experiments, tissue processing and assays

Amanda Chipman
Surgical resident
6.0 Calendar months
Assisting with animal experiments and tissue processing and assays

Christine Cavanaugh
Research Assistant
4.8 calendar months
Assisted with animal experiments and tissue processing

Shibani Pati
Co-investigator
0.78 calendar months
Performing assays on lung tissue, blood and bronchoalveolar fluid

Daniel Potter
Research Associate
0.9 calendar months
Performing assays on lung tissue, blood and bronchoalveolar fluid

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

7. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (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.*

8. 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.*

Dried Plasma to Improve Outcomes in Polytrauma, Hemorrhage, and Trauma-Associated Sepsis (TAS): Novel Solutions for the Prolonged Field Care Environment
Grant Log # DM160146
W81XWH-17-2-0054

PI: Dr. Rosemary Kozar **Org:** University of Maryland **Award Amount:** $1,101,644.00

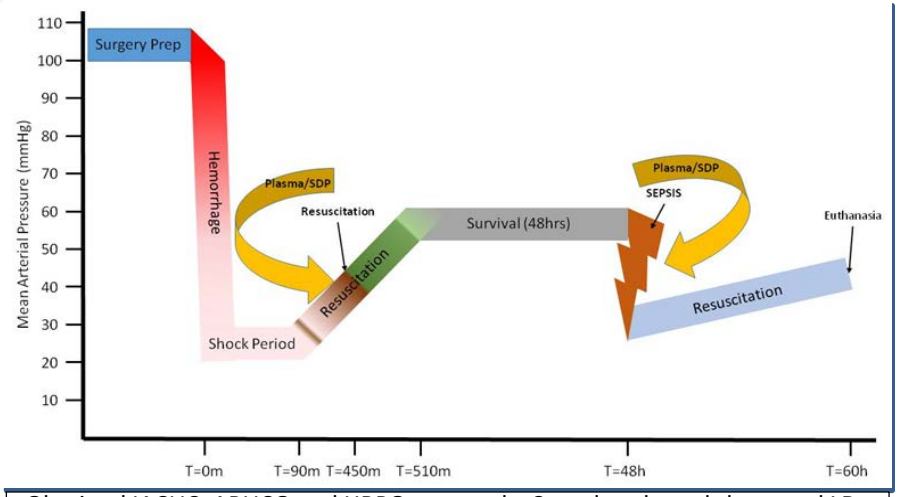
Study/Product Aim(s)

- **SA1. To determine the effects of freeze dried plasma (FDP) compared to fresh frozen plasma (FFP) and hextend on systemic, vascular, organ-specific pathophysiology and survival in a rodent model of hemorrhagic shock (HS) and prolonged hypotensive resuscitation(PHR) with trauma associated sepsis (TAS).**
- **SA2. To determine the modulatory effects of FDP compared to hextend on hemodynamics, end-organ function, coagulopathy and survival in a swine model of TAS.**

Approach

SA1.Mouse model of HS and PHR then resuscitation with FDP, FFP or hextend compared to shams then HS and PHR followed by TAS and resuscitation with similar fluids.

SA2. Swine model of HS and PHR then TAS and resuscitation with either FDP or hextend.



Obtained IACUC, ARUCO and HRPO approvals; Completed cecal slurry and LD 100 experiments; completed short term HS and prolonged field resuscitation surgeries and are working on sample analysis

Timeline and Cost

Activities / CY	17	18	19	20
Aim 1 approvals and slurry/LD100				
Aim 1 HS and PHR surgeries and analysis				
Aim 1 TAS surgeries and analysis				
Aim 2 approvals, surgeries and analysis				
Estimated Budget ($K)	**$451**	**$493**	**$157**	**$000**

September 30, 2018

Goals/Milestones

CY17 Goal – Mouse approvals

CY18 Goals – Mouse HS and PHR

☐ x Complete cecal slurry and LD 100 experiments

☐Complete HS and PHR experiments and analysis mice-experiments done, analysis in progress

CY19 Goal – Complete short term mice experiments, start swine

☐ Complete TAS short term experiments and analysis

☐ Begin swine IACUC and MRMC approvals

CY20 Goal complete mouse and swine studies

☐ Complete mice survival studies

☐ Complete swine studies

Comments/Challenges/Issues/Concerns

- Dried plasma not effective, concern over pH of the product

Budget Expenditure to Date

Projected Expenditure: $451,473 for Year 1

Actual Expenditure: $309,655.23 through 4th quarter

Subcontract to UCSF: Obligated $92,174 for Year 1