AWARD NUMBER: W81XWH-16-7-0066

TITLE: Prothrombin Complex Concentrate for Prolonged Field Carce of War Casualties

PRINCIPAL INVESTIGATOR: Martin Schreiber, MD

CONTRACTING ORGANIZATION: Oregon Health & Science University, Portland, OR

REPORT DATE: January 2023

TYPE OF REPORT: Final

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
January 2023	Final	30Sep2017 - 29Sep2022

4. TITLE AND SUBTITLE

Prothrombin Complex Concentrate for Prolonged Field Care of War Casualties

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-2-0066

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Schreiber, Martin A
email: schreibm@ohsu.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Oregon Health & Science University Portland, OR 97239

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

Patients who initially survive from traumatic thoracic injury are at risk for Acute Respiratory Distress Syndrome (ARDS). The only proven treatments available once ARDS has developed are low tidal volume ventilation (ARDSnet) and proning, but there is no existing treatment strategy to prevent the onset of ARDS following traumatic injury. As a potential solution, recent evidence suggest that prothrombin complex concentrate (Kcentra) acts similarly to plasma to prevent vascular leak and edema, but this has not been investigated in the trauma setting. Therefore, the purpose of this project is to conduct a series of in vitro and in vivo studies to determine if the therapeutic administration of Kcentra prevents the development of ARDS following pulmonary contusion and hemorrhagic shock.

15. SUBJECT TERMS

None Listed

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
U	U	U	UU	14	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

Page

1. INTRODUCTION:

Patients who initially survive from traumatic thoracic injury are at risk for Acute Respiratory Distress Syndrome (ARDS). The only proven treatments available once ARDS has developed are low tidal volume ventilation (ARDSnet) and proning, but there is no existing treatment strategy to prevent the onset of ARDS following traumatic injury. As a potential solution, recent evidence suggest that prothrombin complex concentrate (Kcentra) acts similarly to plasma to prevent vascular leak and edema, but this has not been investigated in the trauma setting. Therefore, the purpose of this project is to conduct a series of *in vitro* and *in vivo* studies to determine if the therapeutic administration of Kcentra prevents the development of ARDS following pulmonary contusion and hemorrhagic shock.

2. KEYWORDS:

Swine, shock, pulmonary contusion, mesenchymal stem cells, acute respiratory distress syndrome, liver injury, endotheliopathy.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major tasks listed in the SOW include:

1) Obtain regulatory approval, run Rat HS model
2) Run mouse model of hemorrhagic shock to test doses of Kcentra
3) Tissue and Molecular Analysis of Rat Model of HS
4) Obtain regulatory approval and test swine model.
5) Run randomized study in swine model of lung injury and hemorrhagic shock
6) Assess blood and tissue samples for inflammation
7) Submit abstracts, publications, and final report to Army

What was accomplished under these goals?

The cumulative achievements for each year of the project are as follows:
Calendar Year 2017: Attain ACURO approval and IACUC approval for all studies
Calendar Year 2018: Perform model development in both rats and swine. Being randomized study in rats and swine. Complete rat experiments.
Calendar Year 2019: Complete swine experiments. Complete analysis of rat samples. Begin running 24-hour model in mice.
Calendar Year 2020: Complete analysis of swine samples. Complete 24-hour mouse model.

Major activities and specific objectives accomplished in this project include:
Major Task #1: Obtain regulatory approval, Rat HS model (UCSF) – 100% complete
UCSF acquired IACUC approval on 10/24/2017 and ACURO approval on 1/10/2018.
Rat model of hemorrhagic shock 100% completed.

Major Task #2: Model of HS, 24 hour survival (Changes planned for a switch from rats to mice) (UCSF) (100% complete)
We received IACUC and ACURO approval to switch from rats to mice for the 24 hour model. Due to COVID-19 stay in place order, we were delayed in starting this study. We ran the 24 hour model in mice and are analyzing the effect of PCC on lung pathology, inflammation and inflammatory gene expression. The salient findings were that endotheliopathy from traumatic injury is prevalent in vehicle treated animals in the acute period (up to 3 hours after injury) and this is mitigated by treatment using fresh frozen plasma. However, at 24 hours post-injury the endotheliopathy is resolved even in the vehicle-treated group (see Figure 2B). This study is appended to this report (Barry et al.) and was published in *Shock*.

Barry M, Trivedi A, Vivona LR, Chui J, Pathipati P, Miyazawa B, Pati S. Recovery of endotheliopathy at 24 hours in an established mouse model of hemorrhagic shock and trauma. *Shock* 58(4):p 313-320, October 2022. PMID: 36256627.

Major Task #3: Tissue and Molecular Analysis of Rat Model of HS (UCSF). 100% completion
We have completed the acute model of HS in rats and have published our work in 2020 (Potter et al.). It is appended to this report. Our work demonstrates critical variability in rodent models for testing of human products. Specifically, there may be xenogeneic responses from the rat model when using human-derived products (See especially Figure 2 and Table 1) which can make some testing inefficacious.

Potter, Daniel R. PhD; Trivedi, Alpa PhD; Lin, Maximillian BA; Miyazawa, Byron Y. BA; Vivona, Lindsay R. BA; McCully, Belinda PhD; Nair, Alison MD; Schreiber, Martin A. MD; Pati, Shibani MD, PhD The effects of human prothrombin complex concentrate on hemorrhagic shock-induced lung injury in rats: Implications for testing human blood products in rodents, Journal of Trauma and Acute Care Surgery: December 2020 - Volume 89 - Issue 6 - p 1068-1075. PMID 32697449.

Major Task #4: Obtain regulatory approval and test swine model (OHSU). 100% completion.
OHSU acquired their most recent IACUC approval on 8/31/2018 and ACURO approval on 10/2/2018.

The model development was completed in October 2018. The details of this model development are described in the 2018 annual report. Additionally, a description of the model is included in the following publication which is appended to this report:

Smith S, Behrens B, McCully B, Murphy J, Bommiasamy A, Goodman A, Dewey E, Pati S, Schreiber M. Aggressive Treatment of Acute Kidney Injury and Hyperkalemia Improves Survival in a Combat Relevant Trauma Model in Swine. *American Journal of Surgery*, 219 (5), 860-864. May 2020. PMID: 32245610

Major Task #5: Run randomized study in swine model of lung injury and hemorrhagic shock (OHSU). 100% completion.
Utilizing our revised protocol developed in 2018, the randomized study was 100% complete as of February 2020. Physiologic data (hemodynamic variables, thrombelastography parameters, blood gases/chemistries) were collected during the protocol and recorded in a database. Plasma and tissue (lung, spleen, kidney, heart) samples were banked for future analysis. Following completion of the experimental work, analysis of the plasma and tissue samples was initiated. The lab at OHSU was closed due to the coronavirus during the spring of 2020.

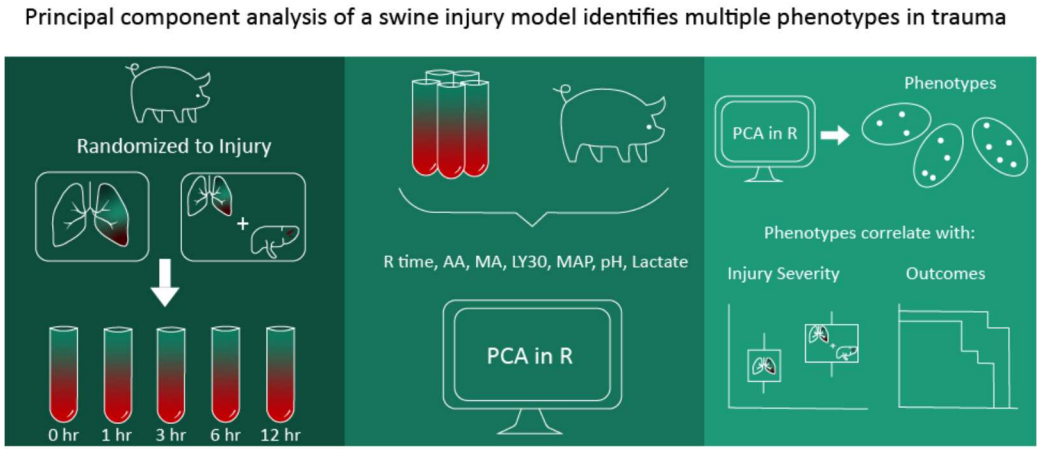
In the 2018 annual report, we reported that the use of an aggressive resuscitation regimen counteracts the development of hyperkalemia following pulmonary contusion and hemorrhagic shock. Dr. Sawyer Smith presented these findings at the 2019 North Pacific Surgical Association meeting. This paper won the Resident Prize for the Best Basic Science paper, and was published in May of 2020 by the American Journal of Surgery.

Dr. Alexandra Dixon presented her abstract on physiological study results, "FFP maintains normal coagulation while PCC induces a hypercoagulable state in a porcine model of pulmonary contusion and hemorrhagic shock", to American College of Surgeons, Committee on Trauma, Region X, winning the top prize for basic science.

Her paper under the same title is appended to this report (Dixon et al.) and was published in 2022:
Alexandra Dixon, Marissa Beiling, Sawyer Smith, Brandon Behrens, Luisa Appleman, Elizabeth Rick, James Murphy, Brianne Madtson, Belinda McCully, Andrew Goodman, Amonpon Kanlerd, Traci Schaller, Sarayu Subramanian, Alpa Trivedi, Shibani Pati, Martin Schreiber. FFP maintains normal coagulation while Kcentra induces a hypercoagulable state in a porcine model of pulmonary contusion and hemorrhagic shock. Journal of Trauma & Acute Care Surgery. 2022 Jul. ;93(1):124-129. PMID: 35261373.

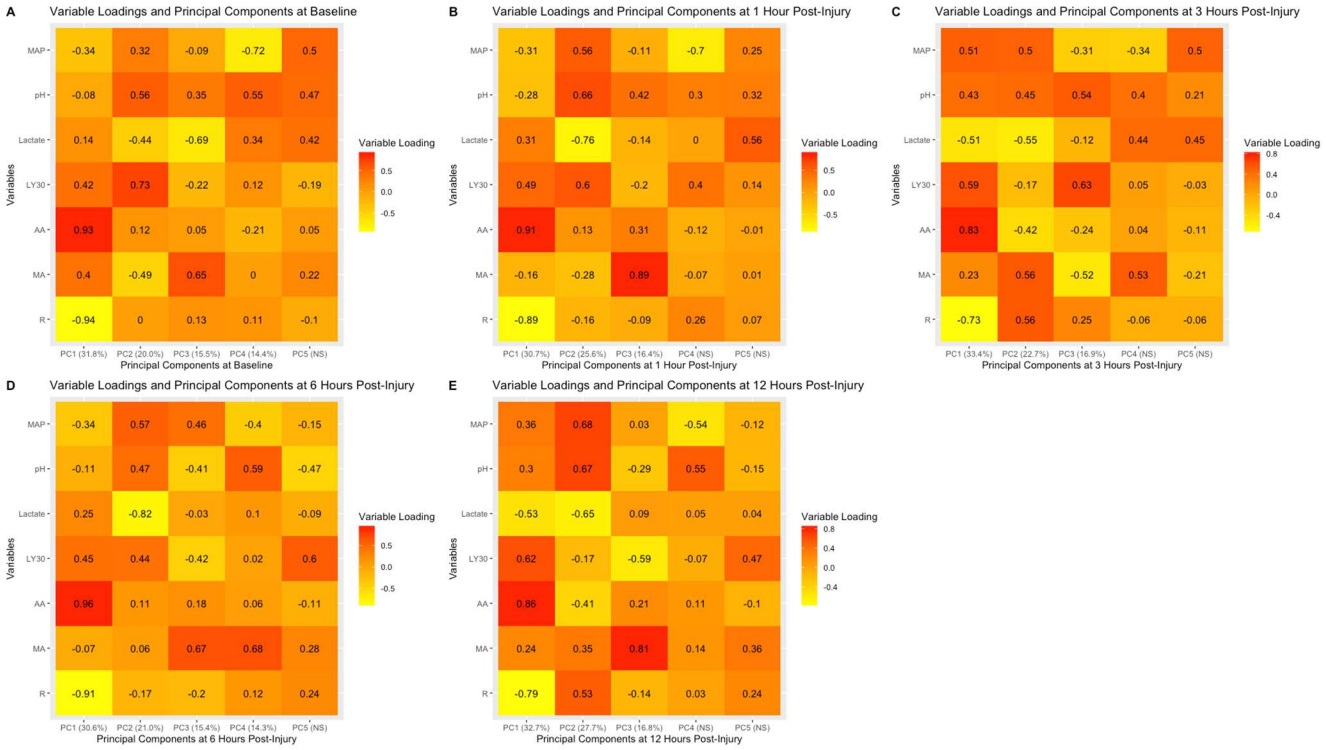
Major Task #6: Assess Blood and Tissue Samples for Inflammation. 100 % completion.
These analyses are completed and additional analysis of trauma phenotypes was recently completed and accepted for publication. The title of the paper is: "Principal component analysis of a swine injury model identifies multiple phenotypes in trauma" and has been accepted to *The Journal of Trauma and Acute Care Surgery*. As it has not yet been proofed, a copy of it cannot be attached here. To summarize the results:

> This study combines laboratory, metabolic, and clinical metrics into an analysis via principal component analysis (PCA) of trauma phenotypes. Some of these phenotypes may correlate with injury severity and may have implications for survival.

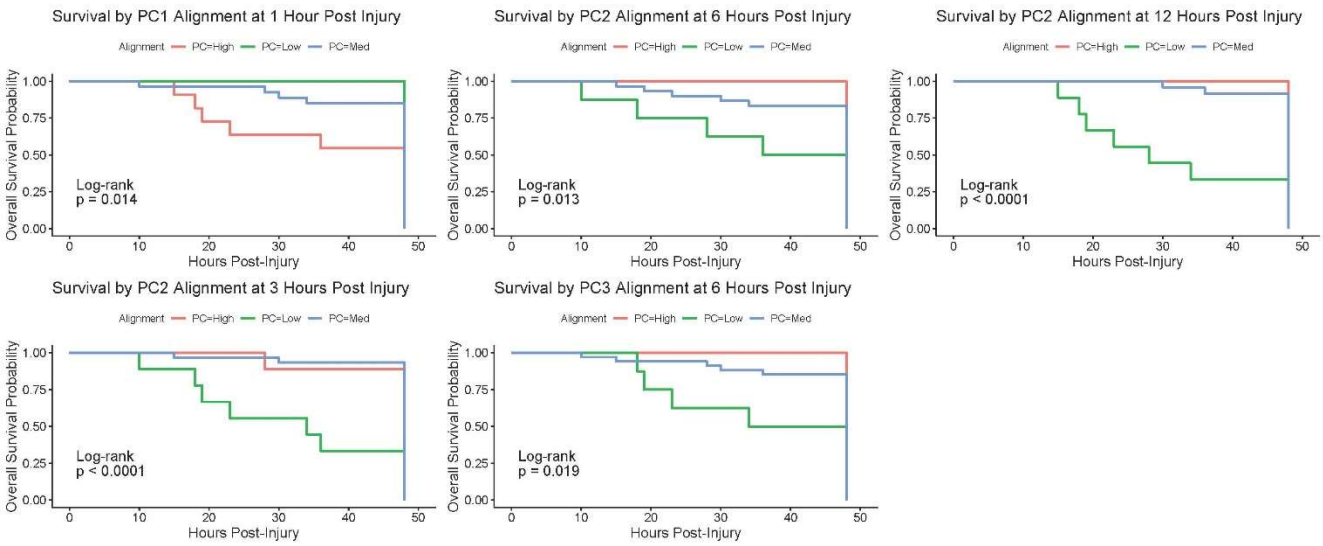


Visual Abstract

SDC 1. Eligibility flow chart for principal component analysis



SDC 3: Control and injured subjects' PCA results at A) Baseline (n = 47). Treatment groups: 13 Vehicle, 14 FFP, 13 KCentra, 7 Control. Injury Groups: 18 pulmonary contusion, 22 pulmonary contusion + liver injury. I 1 hour post-injury (n = 54). Treatment groups: 16 Vehicle, 16 FFP, 16 KCentra, 6 Control. Injury Groups: 25 pulmonary contusion, 23 pulmonary contusion + liver injury. C) 3 hours post-injury (n = 56). Treatment groups: 15 Vehicle, 17 FFP, 16 KCentra, 8 Control. Injury Groups: 24 pulmonary contusion, 24 pulmonary contusion + liver injury. D) 6 hours post-injury (n = 56). Treatment groups: 16 Vehicle, 17 FFP, 16 KCentra, 7 Control. Injury Groups: 25 pulmonary contusion, 24 pulmonary contusion + liver injury.. E) 12 hours post-injury (n = 49). Treatment groups: 14 Vehicle, 14 FFP, 15 KCentra, 6 Control. Injury Groups: 23 pulmonary contusion, 20 pulmonary contusion + liver injury.. Percentages refer to percent variance accounted for by each principal component. NS = Not Significant. MAP = Mean Arterial Pressure. LY30 = Clot Lysis at 30 minutes. AA = Alpha Angle. MA = Maximum Amplitude. R = Reaction time.



SDC 4. Kaplan-Meier Plots of Survival by principal component alignment at 1, 3, 6, and 12 hours post-injury

Additionally, the data resulting from these samples is being analyzed with data from a similar model for generating subsequent manuscripts.

Major Task #7: Submit abstracts, publications, and final report to Army. 90% completion.
With the new analyses shown under Major Task 6, another manuscript has been prepared and is in the submission process. Additionally, because of the wealth of data collected in this longitudinal study we anticipate being able to analyze and utilize it other manuscripts in the future.

What opportunities for training and professional development has the project provided?
Nothing to Report.

How were the results disseminated to communities of interest?
In September of 2020, Dr. Schreiber presented results from the study to MRMC Grant Update Panel.

Additionally, as indicted above, Dr. Alexandra Dixon, presented results from this study and won the basic science competition for Region X, CoT.

What do you plan to do during the next reporting period to accomplish the goals?
The next step for this project is to take our candidate product into a study using human patients that mirror the models used in this project. The lead candidate product, Kcentra, is a biologic product that has already been given FDA approval. So, it already is a tremendous aid to the general population. We aim to expands it purview and demonstrate that it can be an aid to patients with traumatic thoracic injury and hemorrhage.

Follow-on-funding has been secured to implement Kcentra in a pre-hospital setting. The funding source is the Department of Defense office via the Congressionally Directed Medical Research Programs (CDMRP).

4. **IMPACT: What was the impact on the development of the principal discipline(s) of the project?**

We have generated two published reports (Smith et al. and Potter et al.) that we believe have advanced the field of trauma research. The first (Smith et al.) establishes a large animal model and specifically a resuscitation protocol that improves the survival of animals over the course of a 24+ hour experiment. The second (Potter et al.) outlines the caveats of using an animal model for testing human products and the complications that might arise from this difference between humans and the species being utilized in the study.

Additionally, Dixon et al. uncovers important biology that could influence clinical treatment of patients. Specifically, the manuscript shows that Kcentra induces a hypercoaguable state which may be better suited than standard of care for injured persons in remote or austere settings. **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?

We believe we have improved pre-clinical research by refining models for research. This will lead to more streamlined research in our lab and hopefully in other labs utilizing animal models of traumatic injury. Ideally, this will expedite the rise of innovative treatments to the clinical setting which will impact how we treat severely injured patients.

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

We tested markers of endotheliopathy in a mouse model 24 hours after injury. Our hypothesis was not supported. That is, after 24 hours injured animals treated with vehicle were not significantly different from animals treated with standard of care. However, we were still able to utilize these data and produce a publication (Barry et al.) from it.

Changes that had a significant impact on expenditures

Nothing to Report.

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

Significant changes in use of biohazards and/or select agents
Nothing to Report

6. PRODUCTS:

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

- **Journal publications.**
  Potter, Daniel R. PhD; Trivedi, Alpa PhD; Lin, Maximillian BA; Miyazawa, Byron Y. BA; Vivona, Lindsay R. BA; McCully, Belinda PhD; Nair, Alison MD; Schreiber, Martin A. MD; Pati, Shibani MD, PhD The effects of human prothrombin complex concentrate on hemorrhagic shock-induced lung injury in rats: Implications for testing human blood products in rodents, *Journal of Trauma and Acute Care Surgery*, 89(6): 1068-1075. December 2020. PMID: 36256627

  Smith S, Behrens B, McCully B, Murphy J, Bommiasamy A, Goodman A, Dewey E, Pati S, Schreiber M. Aggressive Treatment of Acute Kidney Injury and Hyperkalemia Improves Survival in a Combat Relevant Trauma Model in Swine. *American Journal of Surgery*, 219 (5), 860-864. May 2020. PMID: 32245610

  Barry M, Trivedi A, Vivona LR, Chui J, Pathipati P, Miyazawa B, Pati S. Recovery of endotheliopathy at 24 hours in an established mouse model of hemorrhagic shock and trauma. *Shock* 58(4):p 313-320, October 2022. PMID: 36256627

  Alexandra Dixon, Marissa Beiling, Sawyer Smith, Brandon Behrens, Luisa Appleman, Elizabeth Rick, James Murphy, Brianne Madtson, Belinda McCully, Andrew Goodman, Amonpon Kanlerd, Traci Schaller, Sarayu Subramanian, Alpa Trivedi, Shibani Pati, Martin Schreiber. FFP maintains normal coagulation while Kcentra induces a hypercoagulable state in a porcine model of pulmonary contusion and hemorrhagic shock. *Journal of Trauma & Acute Care Surgery*. 93(1):124-129. July 2022. PMID: 35261373.

Buzzard L, Smith S, Dixon A, Kenny J, Appleman M, Subramanian S, Behrens D, Rick E, Madtson B, Goodman A, Murphy J, McCully B, Kanlerd A, Trivedi A, Pati S, Schreiber M. Principal component analysis of a swine injury model identifies multiple phenotypes in trauma. 2023. Accepted at *The Journal of Trauma and Acute Care Surgery*.

- **Books or other non-periodical, one-time publications.**
  Nothing to Report.

- **Other publications, conference papers and presentations.**
  Nothing to Report.

- **Website(s) or other Internet site(s)**
  Nothing to Report

- **Technologies or techniques**
  Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Nothing to Report.

- **Other Products**
  Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

OHSU
Name: Martin A. Schreiber, MD
Project Role: PI
Nearest person month worked: 1.8 calendar months
Contribution to Project: Dr. Schreiber has provided oversight and day-to-day management of the grant.

Name: James M. Murphy, MD
Project Role: Research Associate/Veterinary Technician
Nearest person month worked: 3.0 calendar months
Contribution to Project: Dr. Murphy is responsible for the designing the anesthesia/sedation regimen, surgical preparation, and overall care of the animals during surgery and recovery.

Name: Alix Dixon, MD
Project Role: Research Resident
Nearest person month worked: 3.0 calendar months

Contribution to project: Dr. Dixon is the lead resident on the project. She prepares and performs the swine surgery, monitors the experiment, organizes data and prepares data for presentation.

Name: Andrew Goodman
Project Role: Coordinator
Nearest person month worked: 6.0 calendar months
Contribution to project: Andrew performs various roles in administration, animal sedation, surgery, and sample processing.

Name: Maria Luisa Appleman, PhD
Project Role: Coordinator
Nearest person month worked: 6.0 calendar months
Contribution to project: Dr. Appleman performs various roles in administration, animal sedation, surgery, protocol management and sample processing.

Name: Brianne Madtson
Project Role: Coordinator
Nearest person month worked: 4.5 calendar months
Contribution to project: Brianne performs various roles in administration, protocol management, treatment preparation, ordering, and sample processing.

Name: Joseph Garay, PhD
Project Role: Senior scientist
Nearest person month worked: 1.5 calendar months
Contribution to Project: Dr. Garay has aided in analysis of samples and tissue. He also leads in administration of the basic science work.

Name: S. James El-Haddi, MD
Project Role: Analyst and writer
Nearest person month worked: 1.0 calendar months
Contribution to project: James has analyzed data produced by the study and written abstracts for submission.

Name: Elizabeth Rick, BS
Project Role: Research Assistant
Nearest person month worked: 1.5 calendar months
Contribution to project: Beth runs the proposed assays for the project.

<u>UCSF</u>
Name: Shibani Pati MD PhD
Project Role- PI UCSF
Nearest person month worked: 1.8 calendar months
Contribution to project: Supervised design and execution of all work and studies. Review data and coordinates groups.

Name: Alpa Mahuvakar, PhD
Project Role: Scientist
Nearest person month worked: 5.4 calendar months
Contribution to project: Involved in planning and execution of studies, coordination with OHSU, running and coordination of in vivo mice experiments.

Name: Byron Miyazawa B Sc.
Project Role: Scientist
Nearest person month worked: 6.6 calendar months
Contribution to project: In vitro assays and in vivo work.

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?
None

8. **SPECIAL REPORTING REQUIREMENTS**
   COLLABORATIVE AWARDS:
   QUAD CHARTS:
   Not applicable

9. **APPENDICES:** Not applicable