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Award Number: W81XWH-15-2-0072

TITLE: Combination of Extracorporeal Life Support and Mesechymal Stem Cell Therapy for Treatment of ARDS in Combat Casualties and Evacuation if Service Members with ARDS

PRINCIPAL INVESTIGATOR: Mauricio Rojas, M.D

CONTRACTING ORGANIZATION:University of Pittsburgh
Pittsburgh PA 15213

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Transfer of injured service members from the Level 3 combat support hospital to level 4 and 5 medical facility increases their chance of survival from devastating injuries. Aeromedical evacuation of patients with Acute Respiratory Distress Syndrome (ARDS) is sometimes beyond the possibilities because of limitations providing ventilator support in flight with a possible further deterioration in patient status. Cell based therapy with adult bone marrow-derived mesenchymal stromal cells (B-MSC) in experimental models of ARDS has been the focus of intense investigation. Data suggest that administered allogeneic B-MSCs can mitigate hypoxemia in ARDS and promote recovery. However, it is unknown how this new form of therapy can be used as an adjunct to current supportive measures for lung failure.

Our objective is to complete a series of preclinical studies in large animal models using extracorporeal membrane oxygenation (ECMO) alone or in combination with B-MSC in sheep and pigs with two different models of ARDS. Hypothesis: standalone B-MSC therapy and combination therapy with B-MSC and ECMO lead to reduction in invasiveness of mechanical ventilation and inflammatory mediators as well as improvement in oxygenation and functional outcome.

We will use two models to assess the efficacy of B-MSCs alone and with ECMO: A sheep model of LPS-induced ARDS (short-term support), and a pig model of burn-induced ARDS (long-term treatment). Human B-MSCs will be generated from a single healthy normal adult donor. We will utilize up to 50 sheep and 40 pigs for the proposed study.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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Unclassified	Unclassified	Unclassified	Unclassified	10	**19b. TELEPHONE NUMBER** *(include area code)*

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1. Introduction

Transfer of injured service members from the Level 3 combat support hospital to level 4 and 5 medical facilities as efficiently as possible optimizes their chance of survival from their devastating injuries1. These types of transport operations involve caring for severely ill young men and women during short-term and long-term transport operations over many hours covering distances from 200 to as far as 8000 miles2. US Air Force Critical Care Air Transport Teams (CCATTs) and the U.S. Army Burn Flight Team (BFT) are an integral component of modern casualty care, allowing early transport of critically ill patients3-5. Aeromedical evacuation of patients with significant pulmonary impairment is sometimes beyond the scope of CCATT and BFT because of limitations of the transport ventilator, potentially exceeding safe ventilator settings in flight and possibly further deterioration in patient status.

A severe complication on critical ill patients is Acute Respiratory Distress Syndrome (**ARDS**) which is defined as acute onset hypoxemia, bilateral radiographic pulmonary infiltrates and lack of atrial pressure hypertension. Emerging evidence suggests that ExtraCorporeal Membrane Oxygenation (**ECMO**) can be a possible therapeutic option for the most severe hypoxemic cases of ARDS. Data from our group and other groups suggests that in the clinical setting ECMO can contribute, by providing “lung rest”, on the mitigation of the severity of ARDS and inducing recovery of the lung6-9.

The goal of this study is to develop clinical practice guidelines for optimal use of stem cell therapies with or without low and high flow extracorporeal life support technologies and in combination with contemporary mechanical ventilation strategies as relevant to en-route care for combat casualties with lung failure. This proposal is the result of a close collaboration between the McGowan Institute of Regenerative Medicine, the Division of Cardiothoracic Transplantation and the Division of Pulmonary, Allergy and Critical Care, at the **University of Pittsburgh** with **Battlefield Health Trauma Research Institute (BHTRI) U.S. Army Institute of Surgical Research (USA ISR)**. We propose to take advantage of this partnership to accelerate and validate the use of ECMO and human Stem Cells in patients with ARDS. Our groups have the infrastructure and the involvement of experienced investigators required to assure successful completion of this project. Finally our team has partnered with **Athersys, Inc.** a biotechnology company specialized in the generation of GMP-grade bone marrow derived adherent stem cells

2. Keywords

- Acute respiratory distress syndrome (ARDS)
- LPS-induced ARDS
- Smoke inhalation injury
- Large animal studies
- Mesenchymal stem cell
- extracorporeal life support
- Therapy

3. Accomplishments

What were the major goals of the project?

Our main hypothesize is that: concurrent use of extracorporeal life support (ECLS) and stem cell therapy will improve functional lung parameters and outcomes in sheep with LPS-induced ARDS (Pittsburgh work on a shorter time scale i.e. up to 24 hours) as well as swine with ARDS due to smoke inhalation and burns (USA ISR work on a 5 day format).

-Specific Aim 1: By using large animal models, determine optimal ECMO settings (low or high flow), in the short term, of a 6-hour model of LPS-induced ARDS (Pittsburgh). Our colleagues at BHTR/USAISR will determine the optimal ECMO settings (low or high flow) in a long-term 5-day model of ARDS due to smoke inhalation and burns in swine.
Milestones: Our goal is to complete all the administrative requirements to initiate the project. Revision of the protocols and completion of the first two groups using High pressure ECMO and low pressure HemoLung (ALung)

-**Specific Aim 2:** (Pre-clinical): To determine the ability of ECMO in combination with cell therapy to reduce the severity of ARDS in sheep and pig in both models and in both centers.
Milestones: Our goal is to review of the protocols and completion of the last two groups using High pressure ECMO and low pressure HemoLung (ALung) in combination with the cell therapy

-Specific Aim 3: To determine the safest combination of cell therapy and ECMO, by evaluating the two models of ECMO with MAPCs.
Milestones: Processing of the biological samples collected, analysis of the data and definition of the most appropriate protocol to reduce the severity of ARDS

Accomplishments

We have completed all the proposed experiments. We in the process to analyze the data generated during the experiments and by processing all the samples collected.

Reportable Outcomes

- Standardization of the protocols using large animals as models of ARDS.
- Changes in pulmonary and cardiac activity as consequence of the induced ARDS and the consequences of the different interventions that were proposed in the present application.

Progress Detail

1. Experimental Groups:

This is the list of the total number and dates of the animals used until 07-30-18:

LPS			
#	**Experiment #**	**Date**	**Notes**
1	S2016-01	1/6/2016	Low 100% entire
2	S2016-04	3/3/2016	Sheep died at T4 Low 100% Entire
3	S2016-06	3/22/2016	Low 100% entire
4	S2016-07	4/7/2016	Low 100% entire
5	S2016-08	4/12/2016	Low 100% entire
6	S2016-10	8/2/2016	high 50% before 100% Entire
7	S2016-20	11/30/2016	high 50% before 100% Entire
8	S2016-21	12/7/2017	high 50% before 100% Entire chronic lung infection on path
9	S2017-22	1/11/2017	high 50% before 100% Entire
10	S2017-23	1/24/2017	low 50% before 100% Entire
11	S2017-24	2/8/2017	Sheep died at T5 low 50% before 100% Entire

#	**Experiment #**	**Date**	**Notes**
1	S2016-11	8/2/2016	high 21% with 100% X10min
2	S2016-12	8/9/2016	high 21% with 100% X10min
3	S2016-13	8/17/2016	Sheep died at T3 high 21% with 100% X10min
4	S2016-14	8/24/2016	Sheep died at T5 high 21% with 100% X10min
5	S2016-16	9/7/2016	High 50% before, 100% Entire
6	S2016-17	9/21/2016	High 50% before, 100% Entire
7	S2016-18	9/28/2016	High 50% before, 100% Entire
8	S2016-19	10/11/2016	High 50% before, 100% Entire
9	S2017-27	3/8/2017	Low 50% before, 21% on Alung 100% X10 min
10	S2017-28	3/15/2017	Low and high 50% before, 21% on Alung 100% X10 min

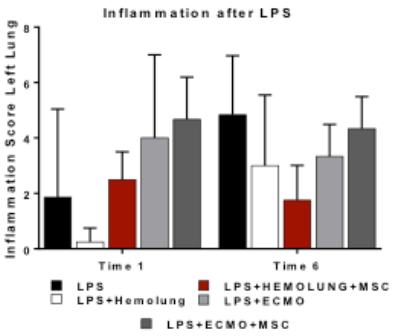
LPS + ECMO			
#	**Experiment #**	**Date**	**Notes**
1	S2017-25	2/15/2017	Low 50% before 21%ECMO Entire
2	S2017-26	22/02/2017	Low 50% before 21%ECMO 100%X10min
3	S2017-30	4/19/2017	Low 50% before 21%ECMO 100%X10min
4	S2017-31	4/26/2017	Low 50% before 21%ECMO 100%X10min

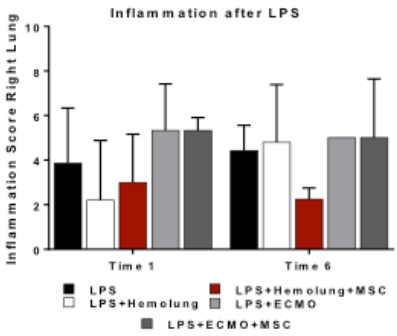
Excluded			
#	Excluded	Date	Notes
1	S2016-02	1/27/2016	Control w/o LPS
2	S2016-03	2/25/2016	Control w/o LPS 100% Entire
3	S2016-05	3/16/2016	Testing animal 100% Entire
4	S2016-09	4/19/2016	Alung w/o LPS 100% Entire
5	S2016-15	8/31/2016	Alung/LPS didn't work 100% Entire
6	S2017-29	4/12/2017	Exp suspended 50% before 100% Entire
7	S2017-32	6/1/2017	SC control, 50% before 100% Entire
8	S2017-33	6/27/2017	Control w/o LPS-Sheep with severe pneumonia

	Experiment #	Date	Notes
34	8/1/2017	S17-34	SALINE
35	8/9/2017	S17-35	LPS
36	8/31/2017	S17-36	LPS+Alung
37	9/12/2017	S17-37	LPS+Alung: Excluded because animal on ARDS at time 0
38	9/14/2017	S17-38	LPS+Alung
39	9/21/2017	S17-39	LPS+Alung
40	9/28/2017	S17-40	LPS+Alung+MSC
41	10/18/2017	S17-41	LPS+Alung+MSC
42	11/1/2017	S17-42	LPS+Alung+MSC
43	11/9/2017	S17-43	LPS+Alung+MSC
44	11/15/2017	S17-44	LPS+ECMO+MSC
45	12/13/2017	S17-45	LPS+ECMO+MSC
46	1/24/2018	S18-46	LPS+ECMO+MSC
47	3/21/18	S18-47	LPS+ECMO

Experimental Results:

Figure 1: Lung Inflammation.





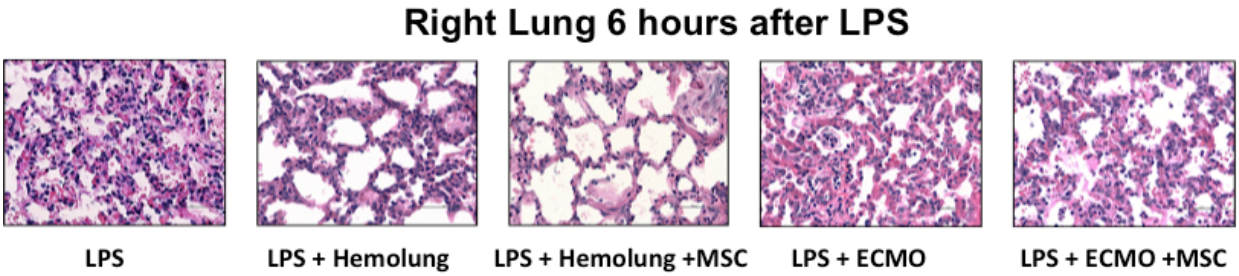


Figure 1. to determine the level of inflammation, using tissue sections collected during the experiments we generate histological sections and stained with Hematoxilin and eosin to determine lung structure, edema and recruitment of inflammatory cells. Six pictures per slide, per animal at each time point where tissue samples were collected. All the samples were scored from 1-7 according to the severity of the injury. Each section was analyzed by the same investigators and it was completely blind about the type of intervention or time point.
A. Clearly, the group that was treated with Hemolung and MSCs has les inflammation one and six hours after administration of LPS. **B**, A representative picture of each group n=20 minimum per group.

Figure 2: Oxygenation

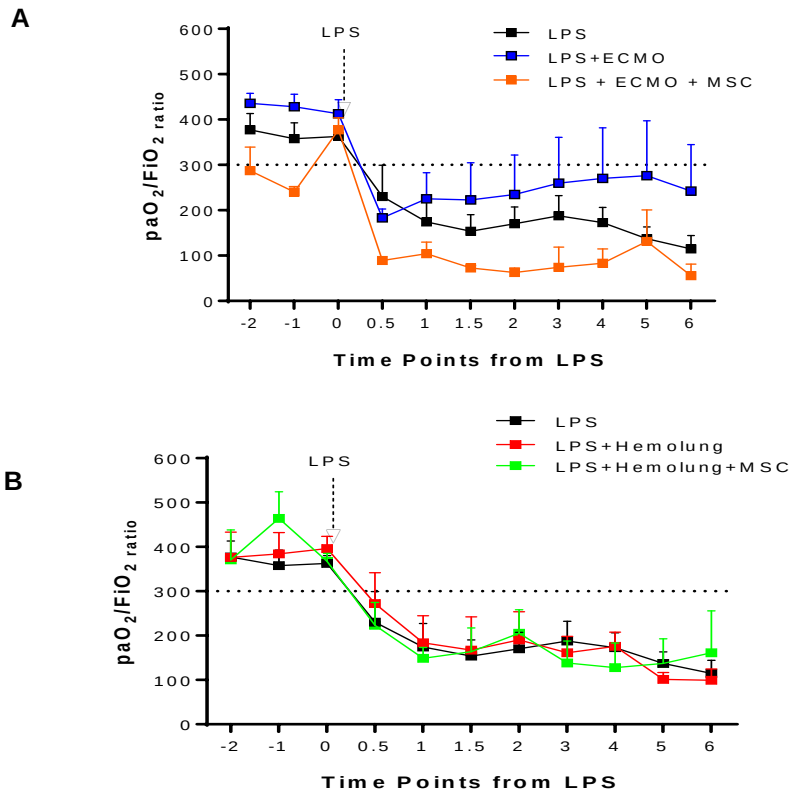


Figure 1. Inclusion of MSCs in the protocol of treatment of ARDS increases efficiency or respiratory devices. We evaluated oxygenation on sheep with LPS-induced ARDS with respiratory support of ECMO (**A**) or Hemolung (**B**) without or with intratracheal infusion of MSCs. We observed that in both protocols the animals treated with the combination device and MSCs performed similar than device alone.

Figure 3: Cardiac Function

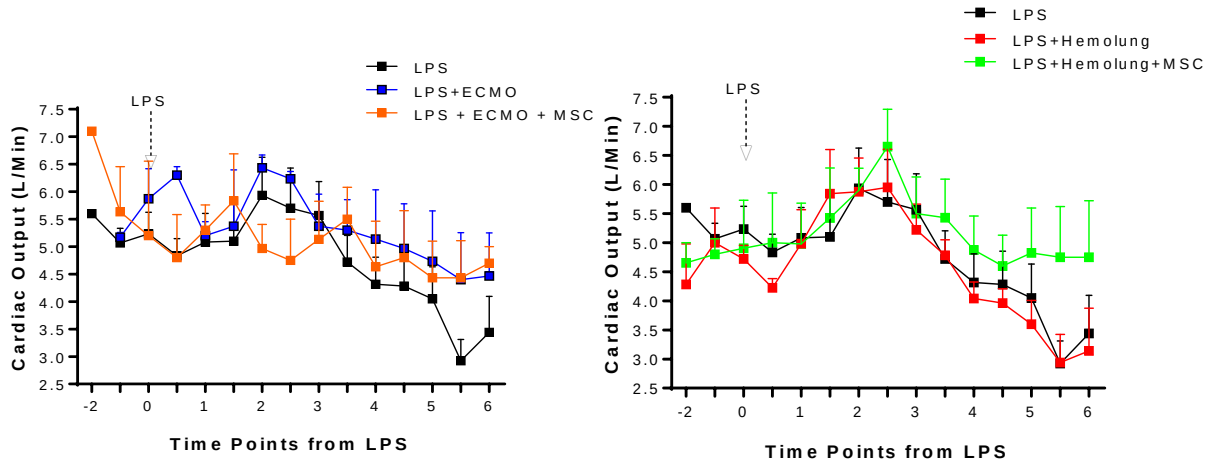


Figure 2. Use of lung devices and their consequences on cardiac function. Decrease on cardiac function had been a main complication on animals with LPS-induced ARDS. We observed that in the group treated with ECMO animals had the strongest cardiac output, independent of the use or not of cells. Contrary in the group of hemolung -MSCs were able to rescue cardiac output.

Figure 4: Mitochondrial Activity

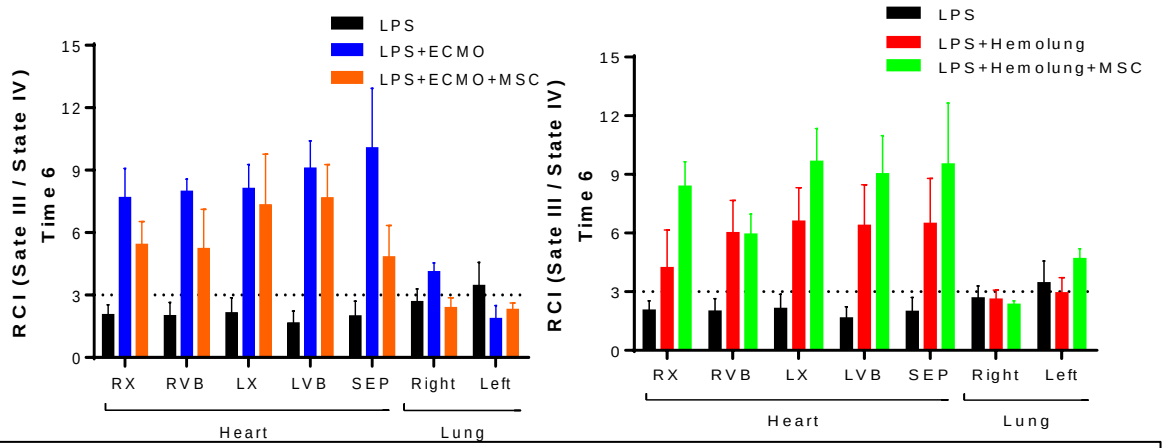


Figure 3. We correlated cardiac function with bioenergetics in the heart by measuring mitochondrial activity at the end of the experiment. We observed a significant increase on mitochondrial function on animals with a lung device and MSCs. These results correlate with cardiac output data.

Figure 5: Oxygenation by ECMO and HemoLung (Alung)

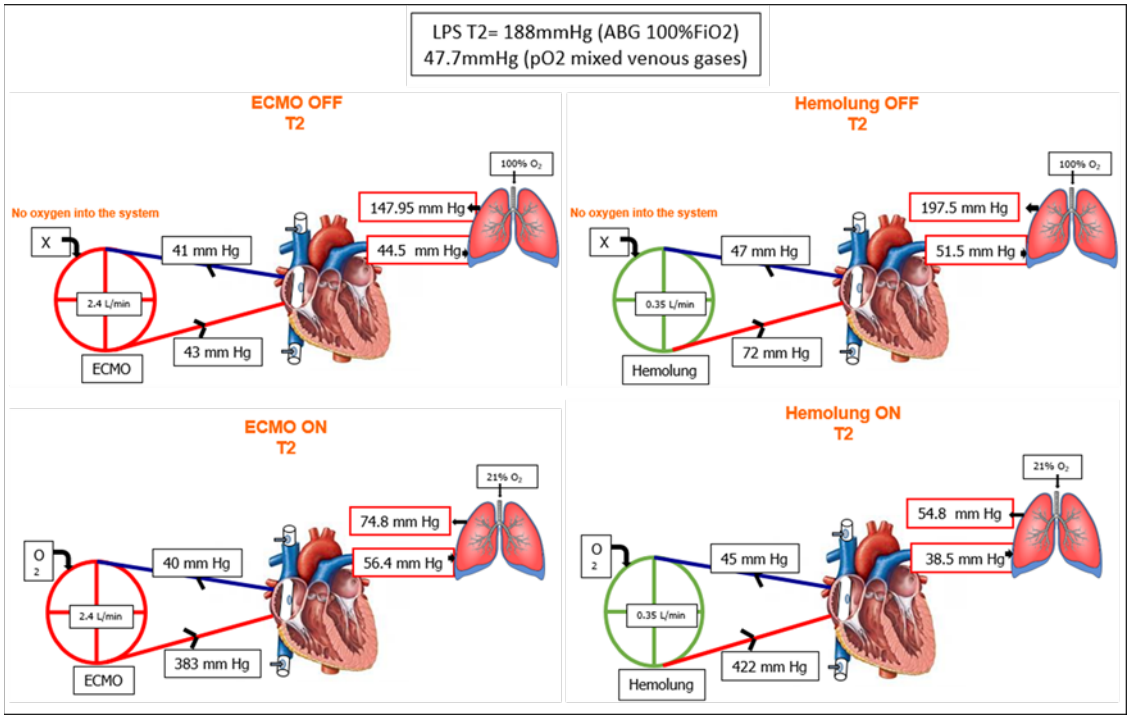
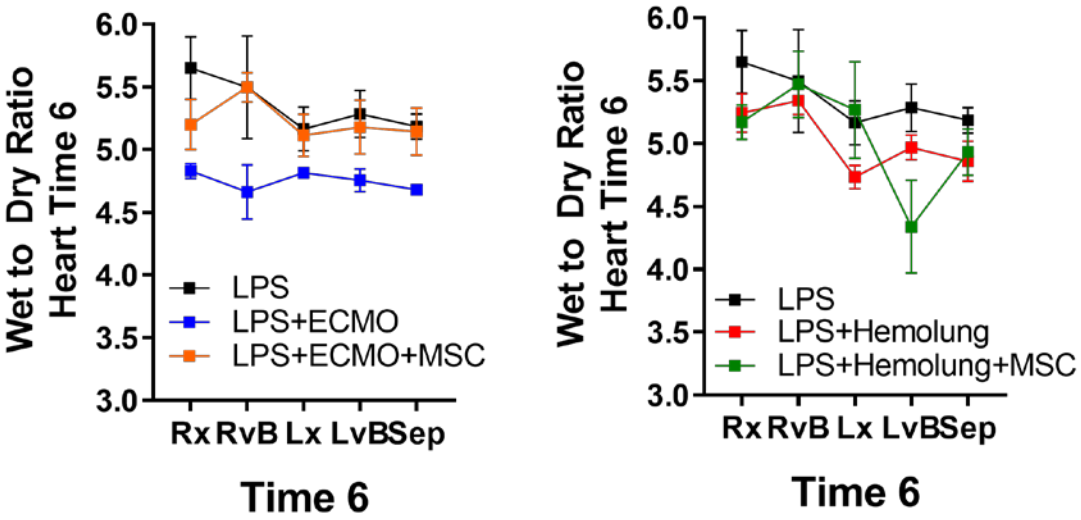


Figure 6: Measure of heart injury: A) Heart Edema and changes in B) Ejection Fraction

A. Wet to Dry



B. Ejection fraction RV and LV.

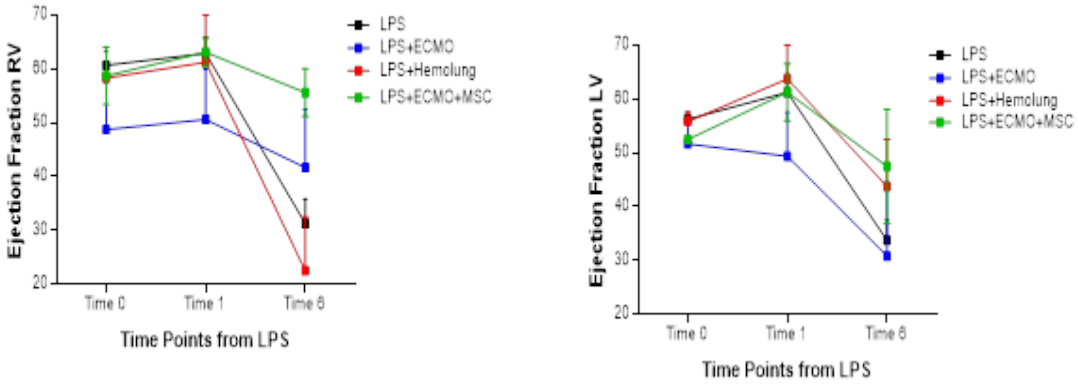


Figure 2. One of the consequences of ARDS and subsequent hypoxia is decrease on heart activity. We measure **A**. water content in the heart which correlates with heart edema and inflammation and **B**. Cardiac contractility by ejection fraction. In injured hearts the ejection fraction decreases.

4. Future Plans

We are processing all the samples collected. This is an update of the status of the processing of all the samples:

Plasma free Hb: Ready to be analyzed.

Isolated RNA from 203 samples from both lungs, three time points Completed.

100 samples cryopulverized from the heart. 70 still pending. RNA isolation: Pending.

.

5. Problems/Issues:

a. Current Problems/Issues

N/A

b. Anticipated Problems/Issues

N/A

6. Personnel Effort

Provide names of current staff along with their roles and percent effort of each on this project. Add additional rows if necessary to list the complete l team. If there is more than one project on this award, breakdown according to each project (one table per project).

Personnel	Role	Percent Effort
Mauricio Rojas	PI	38%
Jonathan D'Cunha	Surgeon	0%
Ergin Kocyildirim	Surgeon	0%
Tomas Drabek	Anesthesiologist	0%
Ron Poropatich	Pulmonologist	0%
Nayra Cardenes	Coordinator	0%
Brian Kimball	Lab Technician	75%
Kentaro Nora	Perfusionist	0%

7. Protocol and Activity Status

For awards involving the use of human subjects, use of human cadavers, and/or use of animal subjects, prepare a summary in accordance with the following subsections. For all other awards, including those involving the use of human anatomical substances (such as tissue or cells or identifiable private information), mark as directed below.

a. Human Use Regulatory Protocols

N/A.

b. Use of Human Cadavers for RDT&E, Education or Training

N/A.

(c) Animal Use Regulatory Protocols

We got approval from our institutional IACUC to include more animals and have two new groups **(**Protocol #: 15034837 Modification #: IM-15034837-37 PHS Assurance Number: D16-00118). Modification was submitted and recently approved by ACURO.

AWARD NUMBER: 0043677 (411381-1)
(Subaward to Geneva for Grant W81XWH-15-2-0072)

TITLE: Combination of Extracorporeal Life Support and Mesenchymal Stem Cell Therapy for Treatment of ARDS in Combat Casualties

PRINCIPAL INVESTIGATOR: Andriy Batchinsky, MD

CONTRACTING ORGANIZATION: The Geneva Foundation

REPORT DATE: Revised 29 November 2018; previously submitted 16 November 2018

TYPE OF REPORT: Annual (30 September 2017 – 29 September 2018)

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

DISTRIBUTION STATEMENT: A

- **INTRODUCTION:**

This report serves as a periodic review of activities and progress made by Dr. Andriy Batchinsky, Principal Investigator at The Geneva Foundation, towards completion of work sub-awarded to The Geneva Foundation as part of federal grant W81XWH-15-2-0072, titled "Combination of Extracorporeal Life Support and Mesenchymal Stem Cell Therapy for Treatment of ARDS in Combat Casualties". This work aims to evaluate new combined therapies of extracorporeal life support (ECLS) and stem cell administration for the treatment of acute respiratory distress syndrome (ARDS) due to inhalation of smoke and 40% body surface area deep burns (further mentioned as "injury") in swine. This animal model will be used to compare 3 groups of injured animals: a group receiving ECLS alone; a group with ECLS combined with stem cell delivery; and a group without specific treatment; all in a clinically relevant, prolonged field care combat casualty care scenario with 72-hour study duration. Successful treatment will improve lung function after ARDS. ECLS treatment is expected to reduce the burden on the mechanical ventilator and partially replace lung function. In the other group, animals will receive ECLS and stem cells which will reduce inflammation and improve immediate function in the lung. The two treatment groups will be compared to animals that receive the same injury and no treatment other than conventional mechanical ventilation.

- **KEYWORDS:**

Smoke inhalation injury; acute respiratory distress syndrome; mesenchymal stem cell; extracorporeal life support

- **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

Specific Aim 1(specified in proposal)	**Timeline**	**Site 1**	**Site 2**
Major Task 1	Months		
Visit of the group of investigators from Pittsburgh to San Antonio to meet with the team at the San Antonio site, to coordinate all future experiments and to ensure that every experiment will be conducted at each site using a similar protocol.	1	Dr. Rojas Dr. D'Cunha Dr. Poropatich	Dr. Batchinsky
We will meet all the members of the team, including cardiothoracic surgeons, perfusionist, MDs,PhDs and technicians to review in detail the protocol at each time point in which all the blood samples, tissue samples, BAL are going to be collected, stored and preserved. Review in detail the surgical protocol, including cannulations, ECMO/ALung, dose and route of administration of endotoxin, stem cells and duration of the protocol. Review the post-surgical analysis of the samples. Histological analysis of formalin fixed, frozen sections, expression of cytokines in lung tissue by RT-PCR, protein expression by western blots, Vibrotome sections of harvested tissues. Cell count of BAL, wet/dry for pulmonary edema. Creation of data bases were information blood analysis will store for future analysis	2	Dr. Rojas Dr. D'Cunha Dr. Poropatich Dr. Kocyildirim Dr. McVerry Dr. Tedrow Dr. Cardenes Dr. Alvarez Mr. Sembrat	Dr. Batchinsky
We will order all the systems and material required to complete the first group of animals or Group #1	2	Dr. Rojas Dr. Cardenes Dr. Kocyildirim Dr. Sembrat	Dr. Batchinsky
Milestone(s) Achieved: Our goal is to complete all the requirements and preparation we need to initiate the project.	2	Dr. Rojas	Dr. Batchinsky

We will do our first preparation only when all the previous subtasks are completed. This will minimize the risk of major problems that can affect our interpretation of the data			
Local IACUC at the university of Pittsburgh have been approved	1	Dr. Rojas	
Milestone Achieved: HRPO/ACURO is approved	1	Dr. Rojas	
Local IACUC at the university of Pittsburgh have been approved	1		
Milestone Achieved: HRPO/ACURO is approved	1		Dr. Batchinsky
Major Task 2			
During the following 2 years our goal is to complete a full preparation every week. According to our plans we will do 25 animals per year. During the 2-3 first preparations of each group we will take a week to analyze the data, evaluate the experiment and decide if there is any suggested change in the protocol that can improve the quality of the preparation and the data collected. ISR: Experiments will be carried out on a weekly basis with an interim analysis half way through the study	2-26	Dr. Rojas Dr. D'Cunha Dr. Kocyildirim Dr. McVerry Dr. Tedrow Dr. Cardenes Dr. Alvarez Mr. Sembrat	Dr. Batchinsky
By using large animal models, determine optimal settings in the short term, of a 6-hour model of LPS-induced ARDS.		Dr. Rojas	
ISR: By using large animals with ARDS due to smoke inhalation and burns we will determine optimal ECCO2R (low flow ECLS) settings in the setting of multi-day ICU care			Dr. Batchinsky
Our goal is to complete all the proposed groups ***Short term ARDS model: 6 hours in sheep*** **1 LPS-ARDS** (*n=10)*	3-7	Dr. Rojas Dr. D'Cunha Dr. Kocyildirim Dr. McVerry Dr. Tedrow Dr. Cardenes Dr. Alvarez Mr. Sembrat	
Specific Aim 2			
Major Task 1 **To determine the ability of ECMO to reduce the severity of ARDS in sheep** **ISR: studies on reduction of ventilator settings and CO2 removal efficiency in swine with ARDS**			
2 LPS-ARDS + High flow veno-venous double cannulation ECMO *(n=10)* *ISR: ARDS due to smoke and burn + standard of care MV (n=10)*	8-46	Dr. Rojas Dr. D'Cunha Dr. Kocyildirim Dr. McVerry Dr. Tedrow Dr. Cardenes Dr. Alvarez Mr. Sembrat	Dr. Batchinsky
3 LPS-ARDS + Low flow veno-venous single cannulation ECMO *(n=10)*	13-17	Dr. Rojas Dr. D'Cunha	

		Dr. Kocyildirim Dr. McVerry Dr. Tedrow Dr. Cardenes Dr. Alvarez Mr. Sembrat	
Specific Aim 3			
Major Task 3 To determine the safest combination of cell therapy and ECMO, by evaluating the two models of ECMO with MAPCs.			
Subtask 1 LPS-ARDS + High flow veno-venous double cannulation ECMO + intratracheally administered 1x106 cells/kg MAPCs (n=10) ISR: ARDS due to smoke and burn (n=10)	18-46	Dr. Rojas Dr. D'Cunha Dr. Kocyildirim Dr. McVerry Dr. Tedrow Dr. Cardenes Dr. Alvarez Mr. Sembrat	Dr. Batchinsky
Subtask 2 LPS-ARDS + Low flow veno-venous single cannulation ECMO + intratracheally administered 1x106 cells/kg MAPCs 2 ISR: ARDS due to smoke and burn + low flow ECLS + IV stem cells (1x106 cells/kg MAPCs), (n=15)	23-46	Dr. Rojas Dr. D'Cunha Dr. Tedrow Dr. Kocyildirim Dr. McVerry Dr. Cardenes Dr. Alvarez Mr. Sembrat	Dr. Batchinsky
Specific Aim 4			
Analysis of the data, determination of the main conclusions, write the final report and publication of the data	46-48	Dr. Rojas Dr. D'Cunha Dr. Tedrow Dr. Poropatich Dr. Batchinsky Data Manager/Statistician	Dr. Batchinsky

For Dr. Batchinsky's portion of the above aims, he was to conduct three experimental groups in swine: 1) ARDS induced by smoke inhalation and 40% TBSA burn (n=10); 2) ARDS induced by smoke inhalation and 40% TBSA burn treated with low-flow ECLS only (n=15); 3) ARDS induced by smoke inhalation and 40% TBSA burn, treated with low-flow ECLS + MAPCs (n=15).

- **What was accomplished under these goals?**

Previous complications and delays necessitated a move of this project to a new location (Brooks City Base, BCB), which necessitated rebuilding of the infrastructure and capabilities dedicated to this effort. Beginning in September 2017, discussions began with the IACUC at BCB. Between September 2017 and October 2017, a new animal use protocol was written and submitted to the BCB IACUC. This protocol (BPTS 17-07) was IACUC approved in November 2017, and subsequently submitted to ACURO for secondary level approval.

Beginning in October 2017, and continuing through March 2018, a new smoke inhalation system was purchased, built, tested, refined, and validated. This included purchase of equipment, assembly and modification of components, and testing and refinement. The first testing was completed in December of 2017,

and refinement and testing continued through March 2018 in a progressive fashion, first without animals, then with euthanized carcasses to validate the mechanics of the new system.

This new system has routinely achieved wood chip combustion ≥ 80% combustion, which correlates with the legacy system at the USAISR.

During this time period, Dr. Batchinsky invested significant time (beginning in December 2017) on sourcing a new supply of quantities of mesenchymal stem cells in large enough alliquots to facilitate the remainder of this study. His efforts continued through July 2018, when he identified a new vendor that agreed to support this study (BioBridge Global, San Antonio, TX).

On 6 AUG 18, the first animal study was conducted at the BCB location (Animal PITT01). This animal began the study at 58.9 kg, was randomized to the Injured Control group (Group 1 above), received 32 smoke breaths at 30 mL/kg (total smoke dose 54.4 L). We do not have CoHb levels on this animal, as our co-oximeter had not arrived prior to the start of the study. This animal reached acute respiratory distress syndrome (ARDS) 18 hours after injury and survived the entire 72-hour studyduration.

On 11 SEP 18, the second animal study of this period was performed (PITT 02). This animal weighed 49.7 kg at study onset and was again randomized to the Injured Control Group (Group 1 above). This bring the total number of IC animal to date (both ISR and BCB locations) to 5. This animal received 44 smoke breaths at 30 mL/kg, achieving a maximum CoHb of 82.3% with a total smoke dose of 68.2 L. This animal achieved ARDS 24 hours after injury and survived the entire 72-hour study duration.

For Dr. Batchinsky's portion of the overall award aims, he was to conduct three experimental groups in swine: 1) ARDS induced by smoke inhalation and 40% TBSA burn (n=10), 5 completed (3 at ISR and 2 at BCB); 2) ARDS induced by smoke inhalation and 40% TBSA burn treated with low-flow ECLS only (n=15), 5 completed, the remainder to be completed alongside ECLS + MAPCs group in NCE. 3) ARDS induced by smoke inhalation and 40% TBSA burn, treated with low-flow ECLS + MAPCs. To be performed in the new NCE together with 5 more injured controls (group 1). To date we have not had to use any replacement animals which the protocol permitted (n=4).

Animal Tally to date:

Injured Control Group (5/10 complete)

Animal	Date
8580	3 Oct 2016 (ISR)
8953	7 Nov 2016 (ISR)
9716	19 Jun 2017 (ISR)
PITT01	6 Aug 2018 (BCB)
PITT02	11 Sep 2018 (BCB)

ECLS Only Group (5/15 complete)

Animal	Date
8648	1 Nov 2016 (ISR)
9204	14 Nov 2016 (ISR)
9249	18 Jan 2017 (ISR)
9253	31 Jan 2017 (ISR)
7997	6 Feb 2017 (ISR)

Preliminary data from all experiments performed to date:

Major Task 1 and its milestones have been completed see SOW.

Major Task 2.
Specific Aim 1 - USAISR will determine the optimal ECMO settings (low or high flow) in a long-term 5-day model of ARDS due to smoke inhalation and burns in swine (performed between February 2016 and September of 2017. Completed at ISR.

This task included analysis of existing CO_2 removal data and adjunct use of mechanical ventilation in animals that received Hemolung therapy in previous studies in the Batchinsky lab. Using 72 hour round the clock ICU data acquired in injured and uninjured animals treated with Hemolung, we developed a clinical protocol of tidal volume reductions upon initiation of Hemolung therapy after injury. Specifically, we reduce minute ventilation by 50% more due to reduction in tidal volume rather than changes in respiratory rate. The resultant underventilation is mitigated by adjunct use of 500 ml/min flow through the Hemolung which permits significant pre and post membrane CO_2 reduction but also a circa 8% oxygenation benefit See table for pre and post PaO_2 and $PaCO_2$ values (Table 1).

Table 1

		6 Hr	12 Hr	18 Hr	24 Hr	30 Hr	36 Hr	42 Hr	48 Hr	54 Hr	60 Hr	66 Hr	72 Hr
PO2	Pre	33.4 ± 2.42	31.6 ± 1.72	32.8 ± 1.74	33.5 ± 1.5	34.5 ± 2.25	33 ± 2.38	34 ± 4	34.33 ± 1.45	33 ± 2	32.5 ± 0.5	32 ± 0	32.5 ± 0.5
	Post	526.8 ± 21.27	482.6 ± 19.55	482.4 ± 11.05	521.5 ± 13.94	510 ± 26.41	468 ± 24.18	467.67 ± 31.8	499 ± 42	439.5 ± 42.5	406.5 ± 39.5	450 ± 26	497 ± 86
PCO2	Pre	37.58 ± 1.22	36.3 ± 3.29	41.44 ± 3.98	43.78 ± 2.19	43.48 ± 3.58	39.98 ± 2.81	43.9 ± 3.03	44.32 ± 3.21	46.8 ± 2	46.95 ± 1.55	49.8 ± 1.5	59.65 ± 10.25
	Post	23.2 ± 0.83	22.34 ± 0.72	24.78 ± 0.56	24.45 ± 0.64	24.25 ± 1.32	23.48 ± 1.27	25.83 ± 0.64	25.07 ± 0.75	26.35 ± 0.15	26.1 ± 0.3	26.8 ± 0.3	29.8 ± 2.3

Besides this unique data we also developed a nomogram for ventilator setting reductions based on an expected 50% reduction in ventilator settings which has been presented at the Annual International Advances in Therapeutics and Technology: Critical Care of Neonates, Children, and Adults Conference in Snowbird, Utah (presented by Brendan Beely, RRT).

Specific Aim 2 - (Pre-clinical): To determine the ability of ECMO in combination with cell therapy to reduce the severity of ARDS in pigs. Because of unavailability of MSCs we carried out MSC production and characterization between human and swine cells and developed SOPs for optimal storage and administration of cells. We will carry out this work in the NCE. Standard of care group 50% complete (5/10), ECLS treatment without stem cells is 33% complete (5/15). Data from standard of care and ECLS treated animals is provided below.

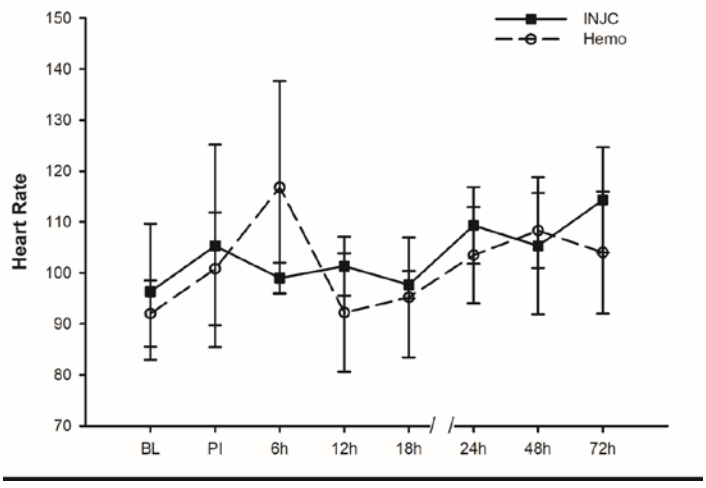


*Figure 1. Heart rate. Solid line, Injured Control; Dashed line, Hemolung. *Hemolung difference vs. baseline. **Injured Control difference vs. baseline. †Between group difference. All statistics $p<0.05$.*

Changes in heart rate were nonspecific between injured controls and Hemolung animals Figure 1.

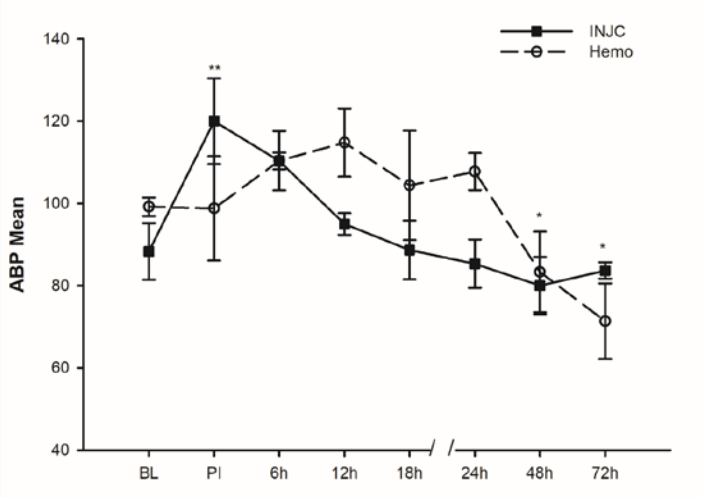


*Figure 2. Mean arterial blood pressure. Solid line, Injured Control; Dashed line, Hemolung. *Hemolung difference vs. baseline. **Injured Control difference vs. baseline. †Between group difference. All statistics p<0.05.*

Similarly, there were no between group changes in mean arterial blood pressure, Figure 2. Within group changes were a minor but significant reduction in blood pressure compared to baseline.

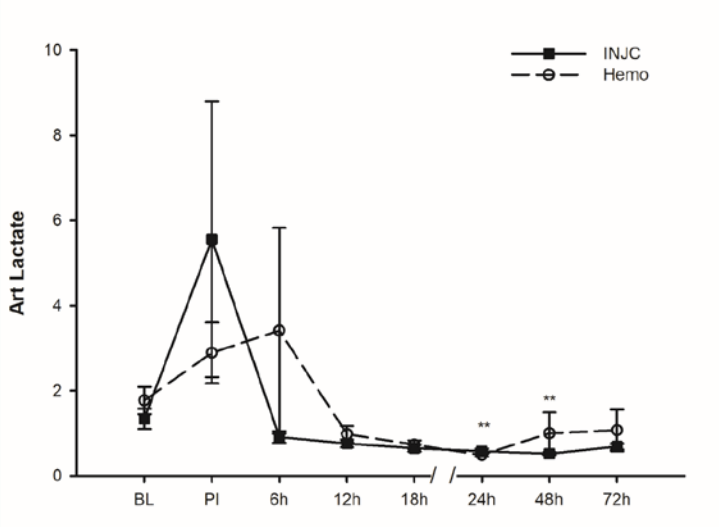


*Figure 3. Arterial lactate. Solid line, Injured Control; Dashed line, Hemolung. *Hemolung difference vs. baseline. **Injured Control difference vs. baseline. †Between group difference. All statistics p<0.05.*

Lactate levels were higher numerically in the injured control group but not different from Hemolung group.

Systemic arterial PCO_2 data is depicted in Figure 4 and showed significantly increase levels in injured controls at 24 and 72 hours vs. baseline values.

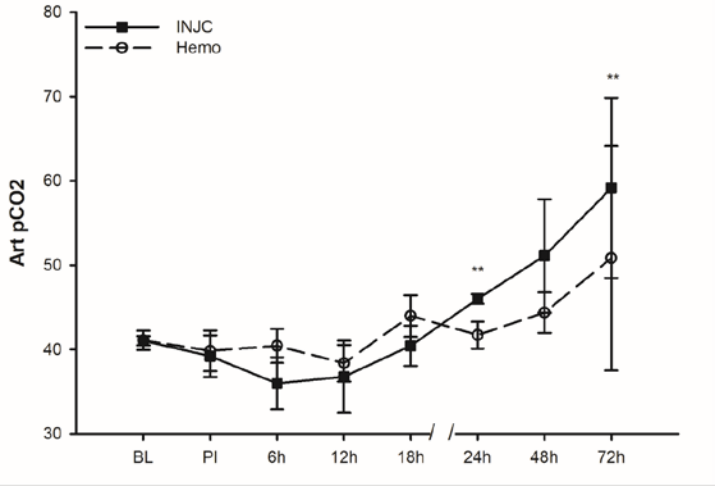


*Figure 4. Arterial PaCO2. Solid line, Injured Control; Dashed line, Hemolung. *Hemolung difference vs. baseline. **Injured Control difference vs. baseline. †Between group difference. All statistics $p<0.05$.*

In the Hemolung group only, pre and post membrane blood gas sampling demonstrated a systematic reduction in $PaCO_2$, See Figure 5.

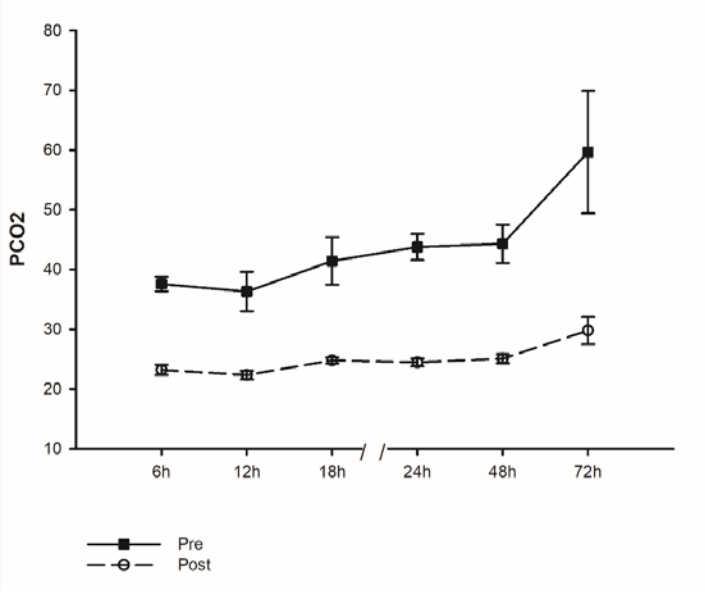


Figure 5. Pre- and post-membrane CO2 measurement. Hemolung group only.

The percent CO2 removal has trended up in Hemolung animals with study progression from an initial 38% removal to 48% by 72 hours, Figure 6.

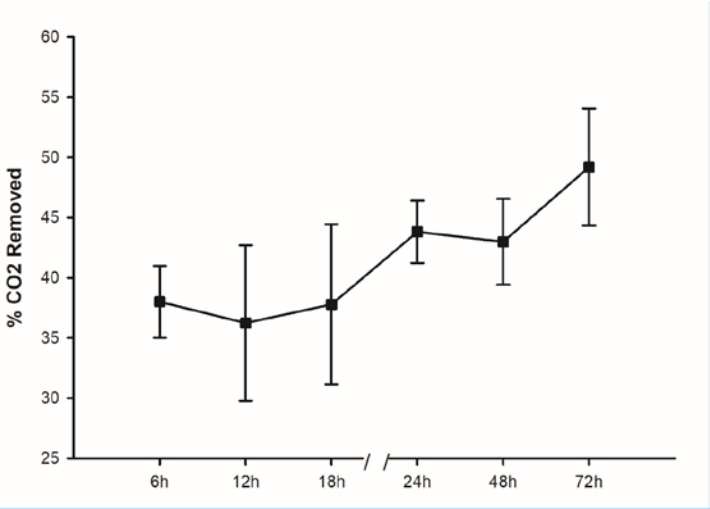


Figure 6. Percent CO2 removal. Hemolung group only.

Injured controls required higher FiO2 settings at 48 and 72 hours vs. baseline, Figure 7.

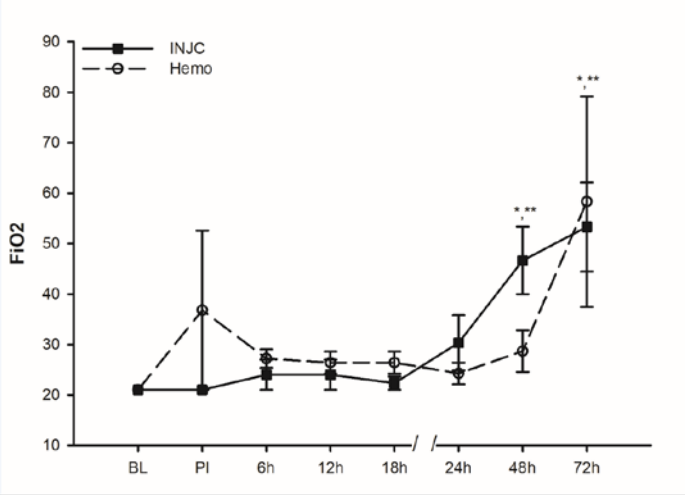


*Figure 7. FiO2. Solid line, Injured Control; Dashed line, Hemolung. *Hemolung difference vs. baseline. **Injured Control difference vs. baseline. †Between group difference. All statistics $p<0.05$.*

Finally, the key ARDs severity index PFR, showed a distinct difference between the injured controls and Hemolung treated animals with more pronounced earlier ARDS in the former and delayed, less severe ARDS in the latter, Figure 8.

The changes in PFR were concomitant with an increasing trend in peak inspiratory pressure reflecting progressive formation of obstructive airway casts- a hallmark of this model, Figure 9.

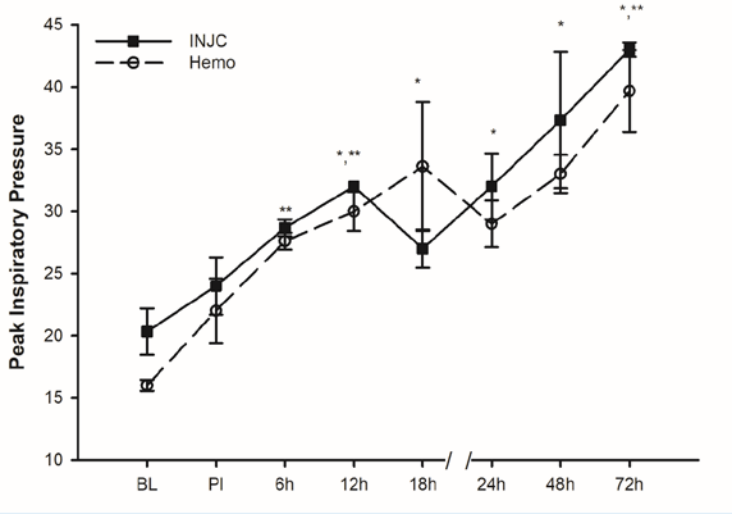


*Figure 8. Peak inspiratory pressure. Solid line, Injured Control; Dashed line, Hemolung. *Hemolung difference vs. baseline. **Injured Control difference vs. baseline. †Between group difference. All statistics p<0.05.*

Perhaps the most intriguing finding in this preliminary analysis is the striking difference in minute ventilation settings between the groups. Compared to the injured controls which stayed at baseline tidal volume settings, Hemolung animals demonstrated a significant reduction in minute ventilation due to the potent CO_2 removal effect of the Hemolung, Figure 10.

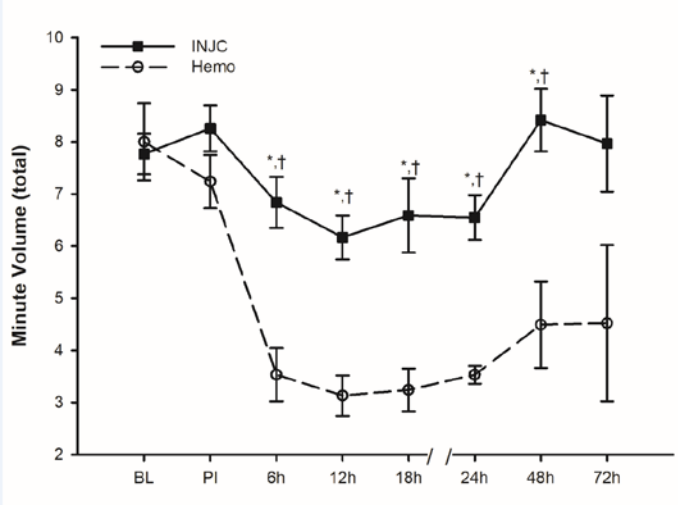


*Figure 9. Minute volume. Solid line, Injured Control; Dashed line, Hemolung. *Hemolung difference vs. baseline. **Injured Control difference vs. baseline. †Between group difference. All statistics p<0.05.*

In addition, respiratory rate was also higher in injured controls compared to baseline whereas it either decreased or did not change in Hemolung animals, Figure 11.

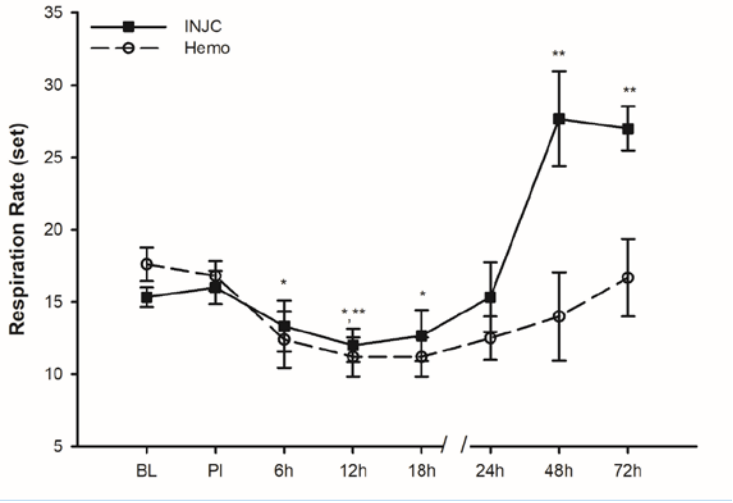


*Figure 10. Respiratory Rate. Solid line, Injured Control; Dashed line, Hemolung. *Hemolung difference vs. baseline. **Injured Control difference vs. baseline. †Between group difference. All statistics $p<0.05$.*

There were no differences in urine output or SPO_2 (data not shown).

In conclusion, this preliminary analysis of experiments performed to date, reflects on the potent CO_2 removal capability of the Hemolung used in adjunct mode to mechanical ventilation as contrasted with inefficiencies of care in injured controls confined to mechanical ventilation alone. These findings, albeit at small n, (3 in Injured Controls and 5 in Hemolung) nonetheless clearly distinguish between the 2 modes of treatment for smoke inhalation and burns and favor earlier utilization of CO_2 removal, in this case via the Hemolung. We anticipate that these differences will be demonstrated even more once we combine MSC and Hemolung therapy.

Major Task 3
Specific Aim 3 - To determine the safest combination of cell therapy and ECMO, by evaluating the two models of ECMO with MSCs.
Will be performed in NCE due to unavailability of stem cells until now.

Specific aim 4 will be performed during NCE.

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

Nothing to report this period.

- **How were the results disseminated to communities of interest?**

Nothing to report this period.

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

Dr. Batchinsky has secured sourcing for the required stem cells to complete this effort. The remaining animal studies are ready to be scheduled and executed in the upcoming final year of performance.

- **IMPACT:**

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

This reporting period saw great improvement in the logistical complexity of the requisite smoke creation and delivery system, producing sustainable, similar results to the legacy USAISR system in a much smaller, mobile footprint. This system was developed and implemented at BCB.

- **What was the impact on other disciplines?**

Nothing to report this period.

- **What was the impact on technology transfer?**

Nothing to report this period.

- **What was the impact on society beyond science and technology?**

Nothing to report this period.

- **CHANGES/PROBLEMS:**

A. Changes in approach and reasons for change

Nothing to report this period.

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

This period saw the location of work on this effort move to a new location. This resulted in some delays and technical need to re-establish lost capabilities from the move, but resulted in a net gain in the ability of the team to execute the work.

The prime delay in the completion of this work, the loss of the stem cell supplier specified in the grant, has been rectified by Dr. Batchinsky this reporting period, and we do not foresee any further delays or complications.

- **Changes that had a significant impact on expenditures**

Nothing to report this period.

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

Nothing to report this period.

- **Significant changes in use or care of human subjects**

Nothing to report this period.

- **Significant changes in use or care of vertebrate animals**

Nothing to report this period.

- **Significant changes in use of biohazards and/or select agents**

Nothing to report this period.

- **PRODUCTS:**

A. List any products resulting from the project during the reporting period.

Nothing to report this period.

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

Scientific output related to or informed by this effort listed below.

I. Journal publications.

Choi J, Chou L, Roberts TR, Beely B, Wendorff D, Espinoza ME, Sieck KN, Dixon AT, Jordan B, S., Brenner M, Chen Z, Necsoiu C, Cancio LC, Batchinsky AI. Point-of-care endoscopic optical coherence tomography detects changes in mucosal thickness in ARDS due to smoke inhalation and burns. Burns 2018; in press.

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

Nothing to report this period.

III. Other publications, conference papers and presentations.

Choi J, Chou L, Roberts TR, Beely B, Wendorff D, Espinoza ME, Sieck KN, Dixon AT, Jordan B, S., Brenner M, Chen Z, Necsoiu C, Cancio LC, Batchinsky AI. Point-of-care endoscopic optical coherence tomography detects changes in mucosal thickness in ARDS due to smoke inhalation and burns. Military Health System Research Symposium. Podium, 2018.

Choi J, Necsoiu C, Wendorff D, Jordan B, Dixon A, Sieck K, Roberts T, Beely B, Cancio L, Batchinsky A. Multiorgan Failure in ARDS: Effects of Adjunct Treatments on End-Organ Damage and Histological Injury Severity. Military Health System Research Symposium. Poster, 2018.

IV. Website(s) or Other Internet site(s)

Nothing to report this period.

V. Technologies or techniques

Nothing to report this period.

VI. Inventions, patent applications, and/or licenses

Nothing to report this period.

VII. Other Products

Nothing to report this period.

- **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

A. What individuals have worked on the project?

Name: Andriy Batchinsky, MD
Project Role: PI
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 4
Contribution to Project: Dr. Batchinsky oversees the execution of this study.

Name: Jae Choi, DVM
Project Role: Research Associate
Nearest person month worked: 2.8
Contribution to Project: Provides anesthesia/sedation, catheterization, instrumentation, sample collection, euthanasia, necropsy. Involved in collection and interpretation of data.
Funding Support: This award

Name: Brendan Beely
Project Role: Research Coordinator
Nearest person month worked: 2.9
Contribution to Project: Provides anesthesia/sedation, catheterization, instrumentation, sample collection, euthanasia, necropsy. Involved in collection and interpretation of data.
Funding Support: This award

Name: Alexander Dixon

Project Role: Laboratory Technician
Nearest person month worked: 2.1
Contribution to Project: Provides anesthesia/sedation, catheterization, instrumentation, sample collection, euthanasia, necropsy. Involved in collection and interpretation of data.
Funding Support: This award

Name: Isabella Garcia
Project Role: Laboratory Technician
Nearest person month worked: 3.6
Contribution to Project: Provides anesthesia/sedation, catheterization, instrumentation, sample collection, euthanasia, necropsy. Involved in collection and interpretation of data.
Funding Support: This award

Name: George Harea
Project Role: Laboratory Technician
Nearest person month worked: 2
Contribution to Project: Provides anesthesia/sedation, catheterization, instrumentation, sample collection, euthanasia, necropsy. Involved in collection and interpretation of data.
Funding Support: This award

Name: Teryn Roberts
Project Role: Research Associate
Nearest person month worked: 6
Contribution to Project: Provides anesthesia/sedation, catheterization, instrumentation, sample collection, euthanasia, necropsy. Involved in collection and interpretation of data.
Funding Support: This award

Name: Kyle Sieck
Project Role: Laboratory Technician
Nearest person month worked: 4.3
Contribution to Project: Provides anesthesia/sedation, catheterization, instrumentation, sample collection, euthanasia, necropsy. Involved in collection and interpretation of data.
Funding Support: This award

Name: Daniel Wendorff
Project Role: Laboratory Manager
Nearest person month worked: 5.9
Contribution to Project: Provides anesthesia/sedation, catheterization, instrumentation, sample collection, euthanasia, necropsy. Involved in collection and interpretation of data.
Funding Support: This award

Name: Robert P. Willis
Project Role: Laboratory Technician
Nearest person month worked: 1
Contribution to Project: Provides anesthesia/sedation, catheterization, instrumentation, sample collection, euthanasia, necropsy. Involved in collection and interpretation of data.
Funding Support: This award

- **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 this period.

- **What other organizations were involved as partners?**

Organization Name: BioBridge Global
Location of Organization: San Antonio, TX
Partner's contribution to the project:

- In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff)
- BioBridge has agreed to supply the needed stem cells for the remaining execution of this study. Agreement reached via Dr. Batchinsky in July 2018.

- **SPECIAL REPORTING REQUIREMENTS**

A. COLLABORATIVE AWARDS

Not applicable.

- **QUAD CHARTS:**

See attached quad chart.

- **APPENDICES:**

Not applicable.