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

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14. ABSTRACT
Transfer of injured service members from the Level 3 combat support hospital to level 4 and 5 medical facilities increase their chance of survival from devastating injuries. The aeromedical evacuation of patients with Acute Respiratory Distress Syndrome (ARDS) is sometimes beyond the possibilities because of limitations providing ventilator support in-flight with possible further deterioration of the patient status. Cell-based therapy with adult bone marrow-derived mesenchymal stromal cells (MSC) in experimental models of ARDS data suggest that administered allogeneic B-MSCs can mitigate hypoxemia and promote recovery. However, it is unknown how this new form of therapy can be used 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 MSC in sheep and pigs with ARDS. Our group had completed the first 19 experiments in which we demonstrated that 3.5 ug/kg of LPS infused i.v. to a sheep induces lung injury equivalent to a moderated ARDS. In the second group of studies, sheep in which a low flow-low pressure ECMO provided respiratory support (ALung) partially rescued the animals returned the parameters of respiratory function to typical values. It is our goal to now use ALung in a combination of MSCs to potentiate their protective effect.

15. SUBJECT TERMS
LPS induced ARDS, ALung, lung injury

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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 increase their chance of survival from devastating injuries. The aeromedical evacuation of patients with Acute Respiratory Distress Syndrome (ARDS) is sometimes beyond the possibilities because of limitations providing ventilator support in-flight with possible further deterioration of the patient status. Cell-based therapy with adult bone marrow-derived mesenchymal stromal cells (MSC) in experimental models of ARDS has been the focus of intense by the investigation. Our previously published data suggest that the administration of allogeneic 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 of this project was to complete a series of preclinical studies in large animal models using a high flow (ECMO) and low flow (ALung) extracorporeal membrane oxygenation devices alone or in combination with bone-marrow-derived mesenchymal stem cells (MSCs) in the ovine model of acute LPS-Induced ARDS. In a separate set of experiments, we explored the consequences of protective ventilation, high PEEP, and standard PEEP without any other intervention.

Simultaneously, our collaborators in the USAISR in San Antonio, TX, under Dr. A. Batchinsky's direction, developed a model in swine of chronic ARDS, they evaluated the use of MSCs in combination with high or low flow extracorporeal lung devices. In Summary, our goal was to use in combination cell therapy and devices like ECMO, leading to a reduction in invasiveness of mechanical ventilation and inflammatory mediators as well as improvement in oxygenation and functional outcome.

Our **hypothesis was**: concurrent use of lung devices 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 6 hours) as well as swine with ARDS due to smoke inhalation and burns (USAISR work on a 5-day format).

2. Keywords

Acute respiratory distress syndrome (ARDS), extracorporeal membrane oxygenation (ECMO), Mesenchymal Stromal Cells (MSCs), transport injured service members.

3. Accomplishments

It is possible to summarize the achievements in two fundamental aspects: Technical objectives and Scientific objectives.

a. Technical Objective:

This study aimed to combine the unique expertise of the BHTRI and the University of Pittsburgh and their experience in these two ARDS models for the following reasons: (1) There are several ARDS types in combat casualties. What works for one clinical setting will not necessarily work in another setting. Smoke and endotoxin-induced ARDS are two extremes of the spectrum concerning pathophysiology. (2) What works for a short-term experiment does not work for a multi-day experiment. (3) Validation of the technologies in two centers will significantly increase their ability to be translated into clinical care. Our final goal was to develop evidence-based clinical practice guidelines for optimal use of low and high flow extracorporeal life support technologies with or without stem cell therapies and contemporary mechanical ventilation strategies relevant to in-theater en-route care for combat casualties with ARDS.

b. What was accomplished under these goals?

We had completed a total of 47 large animal protocols, using the different combinations proposed. It was a technical challenge protocol involving cardiothoracic surgeons, perfusionists, pulmonologists, PhDs, and several postgraduate students and technicians, to supervise the experiment and collect all the information on the physiological variables, collect and process all the different tissue samples. Since the experiment number 10, we had an anesthesiologist dedicated to the study, Dr. Tomas Drabek, an Associated Professor in the Department of Anesthesiology at the University of Pittsburgh. The incorporation of Dr. Drabek increased the reproducibility of the experiment. His main goal was to keep the animal alive with minimal intervention possible, allowing us to compare them.

b.1 Protocol for the Short Term ARDS Model:

In the entire study, we completed 47 non-pregnant female sheep weighing 30 to 50 kg. The animals were randomized into six groups with different therapeutic management protocols described in **Table 1.** The study endpoints were: arterial tension of oxygen; arterial tension of carbon dioxide; the ratio of the partial pressure of oxygen in the arterial blood to a fraction of inspired oxygen; arterial lactate levels; end-tidal CO_2 levels; minute ventilation; pear airway pressure; and post-mortem histology assessment and survival.

i. **Anesthesia:** We followed a standard institutional anesthesia protocol. Each animal was premedicated with intramuscular ketamine (25-30 mg/kg). General anesthesia was maintained with isoflurane (2.5-3%). The animals were ventilated with a mixture of room air and oxygen (fraction of inspired oxygen = 60%) at a frequency of 12 to 15 breaths/min and a tidal volume starting at 10 ml/kg/min initially. The settings were adjusted by maintaining arterial partial

carbon dioxide pressure between 35 and 40 mmHg. During and after the administration of endotoxin, the animals were ventilated with a fraction of inspired oxygen = 100%.

ii. Neck Cannulation: The animals were placed in left lateral recumbency. The right carotid artery is cannulated for recording aortic pressure (AoP) and taking blood samples to analyze blood gases, electrolytes, and pH. A Swan-Ganz catheter was inserted through the jugular vein for recording central venous pressure (CVP), pulmonary artery pressure (PAP), and cardiac output (CO). Necessary intravenous volume substitution was carried out with a saline solution at a rate of 1 ml/kg/min. ***Neither catecholamines nor other hormonal or pressor substances were administered.*** Rectal temperatures and standard peripheral electrocardiographic results will be monitored continuously.

iii. Endotoxin Infusion: All animals receive 3.5 µg/kg *E. coli* endotoxin (Lipopolysaccharide from *E. coli* 055:B5, Sigma L6529) over 30 minutes at 0.7 ml/min to create ARDS (time 0).

iv. Lung Device Support: Veno-venous ECMO was established between SVC and MPA. 1.2-1.4 l/min flows were maintained and ALung at 250 ml/ min. The ventilator settings were adjusted with a tidal volume of 6-7ml/kg and PEEP of 5 cmH2O at a respiratory rate of 10-12/min and room air.

v. Stem Cell Administration: MSCs are an undifferentiated adult stem cell product isolated from bone marrow and expanded in culture to meet clinical dose requirements. The MSCs product (subject to rigorous characterization and product release criteria to ensure consistency and reliability) were cryopreserved and shipped to the treatment site to be inventoried for subsequent use. At the moment of the experiment, MSCs cells were thawed in a water bath at 37oC and washed twice with a 9 ml standard saline solution. We used in the present study a dose of $1x10^6$ cells /kg weigh. After thawing, the MSCs concentration was adjusted to 4 million cells/ml. Cells were administered to the left lower lobe of the animals, one half-hour after the end of the infusion of endotoxin.

vi. Hemodynamic and Blood Gas Studies, Data Acquisition and Analysis: In all groups**,** blood gases and blood sampling were performed before the endotoxin administration and every 60 minutes of the study duration. Hemodynamic measurements, AoP, PAP, CVP, and left atrial pressure (LAP) were monitored with catheter-tip manometers. CO_2 was monitored continuously. All hemodynamic parameters were registered on an HP multi-channel monitor unit and recorded on a personal computer for further offline analysis.

vii. Bronchoscopy and Bronchoalveolar Lavage (BAL): All animals had a BAL before administering endotoxin, 30 min after the end of administration endotoxin, and before the end of the study. Bronchoscopy was performed using a fiberoptic bronchoscope inserted through the endotracheal tube in intubated animals while breathing 100% oxygen. The bronchoscopy was wedged in the left lower lobe, and 30 ml of 0.9% NaCl at room temperature will be instilled and recovered by gentle hand suction.

viii. Histopathologic Evaluation: Lung biopsies were performed from the right and left lower lobes before the end of the study. Tissue sections e stained with hematoxylin-eosin to visualize the structural changes of the lung parenchyma.

viii. Statistics: Statistical analyses were performed on a personal computer with commercially available Microsoft Office 2010 Excel software. All values are expressed as means ± SEM.

ix. Necropsy. The group of performing surgeons performed a gross necropsy. The necropsy included the examinations of the lungs, liver, heart, and heart chambers.

x. ***Clinical Evaluation & Data Points***: In all the groups, peripheral blood samples were obtained at -1, 1, 2, 3, 4, and 5 hours after the initiation of the endotoxin infusion. Blood stored for later measurement of systemic cytokine levels. BAL will be collected but using the right lower lobe at -1 (baseline), 1 hour (before stem cell administration), and the left lower lobe at 5 hours. Complete blood count (CBC), Serum Chemistry, Brain natriuretic peptide (BNP), Activated Clotting Time (ACT), Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT), and Haptoglobin were monitored.

We reviewed the data from each experiment for data analysis and quality control. In case of any level of uncertainty of value, the clinical records were revised during and after each experiment. We also had a bi-weekly meeting, during which we defined implementation or small adjustments in the protocol, like sample collection and detailed review of the clinical records. This resulted in better coordination of the experimental team.

For example, since experiment 11, we measured mitochondrial function on the lung and heart tissue (**Figure 6**). The Clark system allowed us to measure the mitochondrial activity by oxygen consumption during activation. As a consequence of the LPS-induced injury, we observe a decline in mitochondrial function in the heart's specific compartments. Contrary to what was observed in the lung, ALung contributed to a small increase in the mitochondrial activity, suggesting a positive effect of ALung on mitochondrial function.

Our biggest challenge in the present project was that Athersys Inc, the company that was going to give the MultiStem cells for the study because of strategic reasons, decided not to provide us with MultiStem cells. We had demonstrated on previously published studies that MultiStem cells could decrease the severity of LPS-Induced ARDS in sheep. Also, these cells demonstrated in a clinical study their efficacy on patients with ARDS. Instead, they generated normal bone marrow-derived MSCs, which were never used by our group, and we think they are responsible for the final results of the present study.

Table 1

LPS Alone				
#	**Exp**	**Experiment #**	**Date**	**Notes**
1	1	S2016-01	1/6/2016	
2	2	S2016-04	3/3/2016	Sheep died at T4
3	3	S2016-06	3/22/2016	
4	4	S2016-07	4/7/2016	
5	5	S2016-08	4/12/2016	
6	6	S2016-10	8/2/2016	
7	7	S2016-20	11/30/2016	
8	8	S2016-21	12/7/2017	
9	9	S2017-22	1/11/2017	
10	10	S2017-23	1/24/2017	
11	11	S2017-24	2/8/2017	Sheep died at T5

LPS + ALung				
#	**Exp**	**Experiment #**	**Date**	**Notes**
12	1	S2016-11	8/2/2016	
13	2	S2016-12	8/9/2016	
14	3	S2016-13	8/17/2016	Sheep died at T3 high 21% with 100% X10min
15	4	S2016-14	8/24/2016	Sheep died at T5 high 21% with 100% X10min
16	5	S2016-16	9/7/2016	
17	6	S2016-17	9/21/2016	
18	7	S2016-18	9/28/2016	
19	8	S2016-19	10/11/2016	
20	9	S2017-27	3/8/2017	
21	10	S2017-28	3/15/2017	
22	11	S2017-36	8/31/2017	
23	12	S2017-38	9/14/2017	
24	13	S2017-39	9/21/2017	

LPS + ECMO				
#	**Exp**	**Experiment #**	**Date**	**Notes**
25	1	S2017-25	2/15/2017	
26	2	S2017-26	22/02/2017	
27	3	S2017-30	4/19/2017	
28	4	S2017-31	4/26/2017	
29	5	S2018-47	3/21/2018	

LPS +ALung+MSC				
#	Exp	Experiment #	Date	Notes
30	1	S2017-40	9/28/2017	
31	2	S2017-41	10/18/2017	
32	3	S2017-42	11/1/2017	
33	4	S2017-43	11/9/2017	

LPS + ECMO+MSC				
#	Exp	Experiment #	Date	Notes
34	1	S2017-44	11/15/2017	
35	2	S2017-45	12/13/2017	
36	3	S2018-46	1/24/2018	

Controls				
#	Exp	Experiment #	Date	Notes
37	1	S2016-02	1/27/2016	Control w/o LPS
38	2	S2016-03	2/25/2016	Control w/o LPS
39	3	S2016-05	3/16/2016	Testing animal
40	4	S2016-09	4/19/2016	Alung w/o LPS
41	7	S2017-32	6/1/2017	Stem cell control, 50% before 100% w/o LPS
42	1	S2017-34	8/1/2017	Control w/o LPS
43	2	S2017-35	8/9/2017	LPS

Excluded				
#	Exp	Experiment #	Date	Notes
44	5	S2016-15	8/31/2016	Alung/LPS did not work 100%
45	6	S2017-29	4/12/2017	Exp suspended 50% before 100%
46	8	S2017-33	6/27/2017	Control w/o LPS-Sheep with severe pneumonia
47	9	S2017-37	9/12/2017	LPS+Alung: Excluded because animal on ARDS at time 0

c. Scientific objectives:

For the study conducted at the University of Pittsburgh, we had the following main Aims:

-***Specific Aim 1:*** Using large animal models, determine the optimal setting of the extracorporeal lung devices (ALung for low flow or ECMO for high flow), in the short term model of a 6-hour LPS-induced ARDS.

-***Specific Aim 2:*** To determine the safety and efficacy of ALung alone or in combination with cell therapy to reduce the severity of ARDS in sheep with LPS-induced ARDS.

-***Specific Aim 3:*** To determine the safety and efficacy of ECMO alone or in a combination of cell therapy to reduce the severity of LPS-induced ARDS in sheep.

The local IACUAC and ACURO approved all the protocols and the use of large animals for these studies. All the experiments were conducted in the large animal facility of the McGowan Institute for Regenerative Medicine, which is DLAR certificated to carry out this type of experiment.

At the moment of the approval of the present study by the Department of Defense, we had a letter of commitment by Athersys, Inc, where they committed to providing all the MultiStem required to complete the proposed studies. All the preliminary data and experiments included in the original application were completed using MultiStem, which are subpopulations of BM-MSCs that highly effectively modulate inflammation and promote tissue repair. However, at the moment that we requested the cells to complete the proposed studies, Athersys, Inc decided not to give us MultiStem; instead, they produced regular BM-MSCs, which were used for the first time in this model of ARDS.

4. Results:

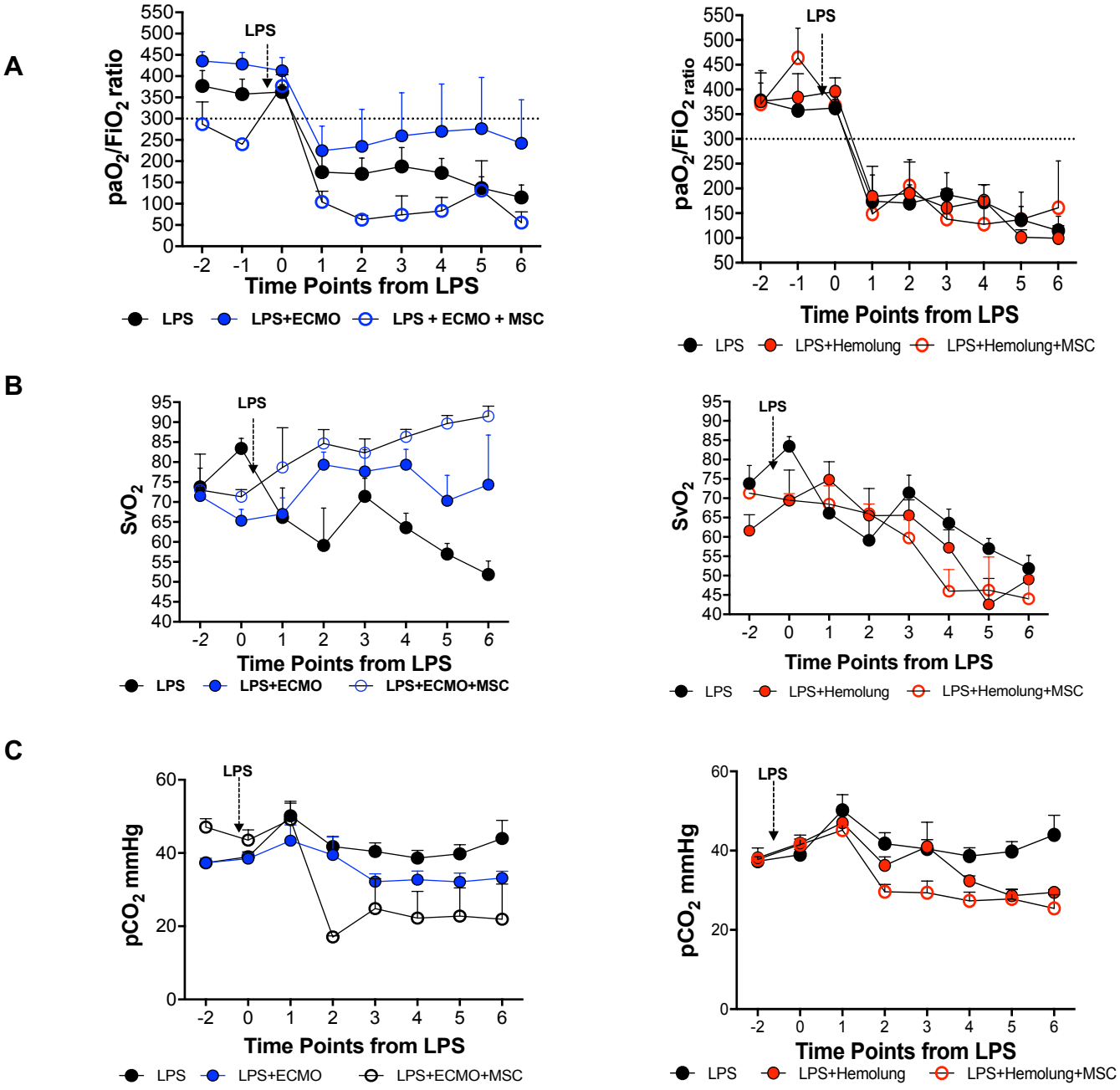

Figure 1. Gas Exchange. We measured different parameters to define gas exchange in our experimental models. Our data suggest that ECMO was the most consistent in maintaining the levels of normal levels of oxygen. Also, the use of MSCs promoted the removal of CO_2. We had compared the effect of ECMO, ALung alone, or in a combination of MSCs.

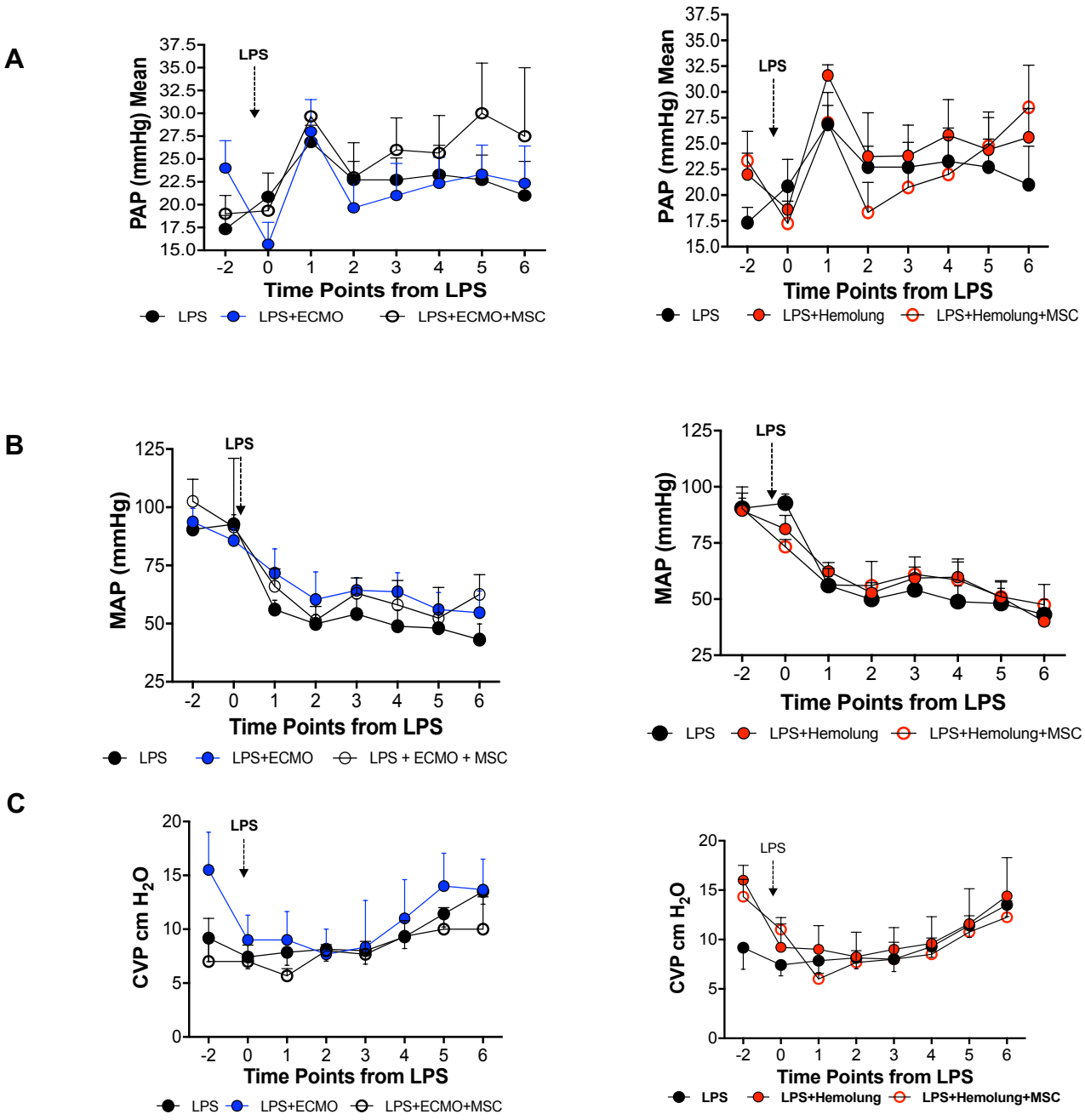

Figure 2. Changes in Hemodynamics. To demonstrate the efficacy of any of the proposed interventions, we collected different hemodynamic parameters during the experiments. A. When pulmonary artery pressure was measured, we observed initial slide protection when MSCs were combined with Alung. We did not observe differences between groups MAP and CVP.

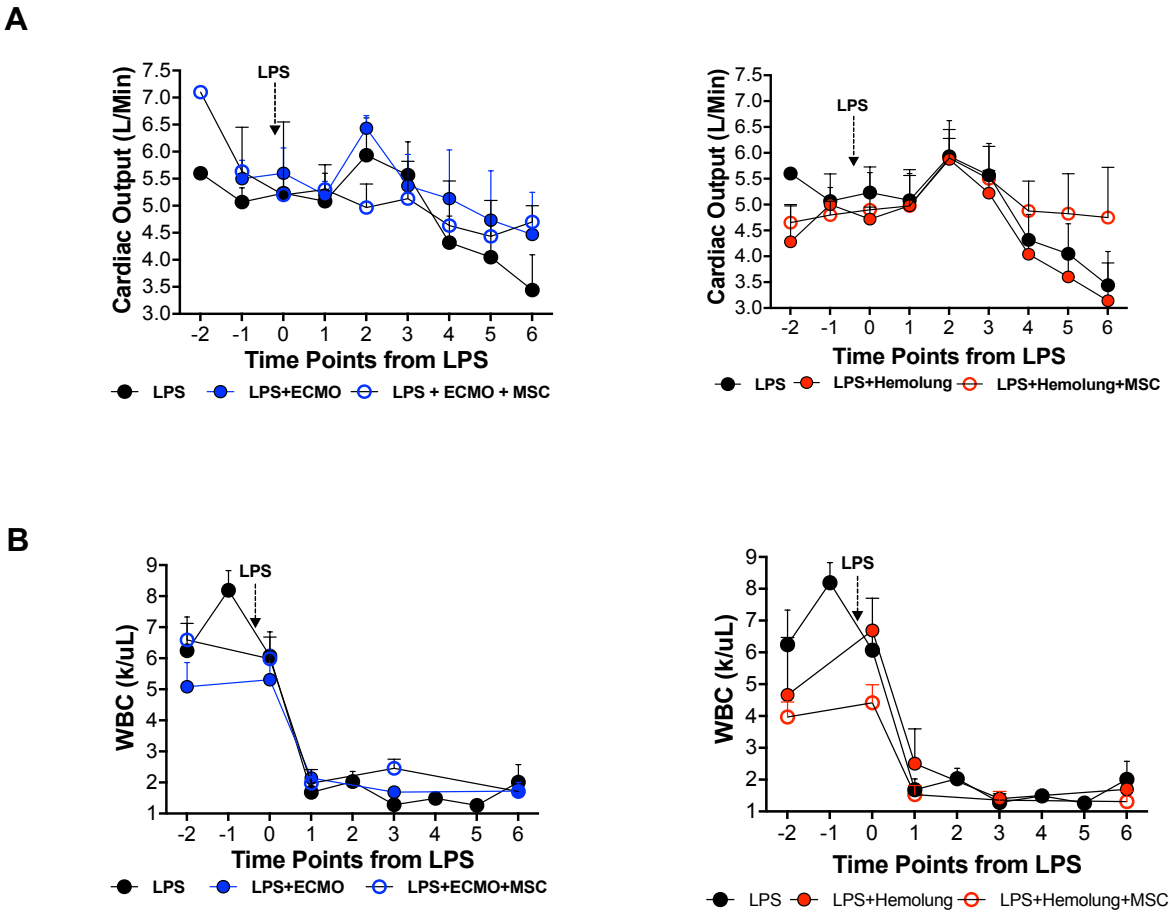

Figure 3. Systemic Effects. We observed the preservation of cardiac output when MSCs were used independently of the device—suggesting a more systemic effect of MSCs. We did not observe any effect on the leukopenia induced during ARDS.

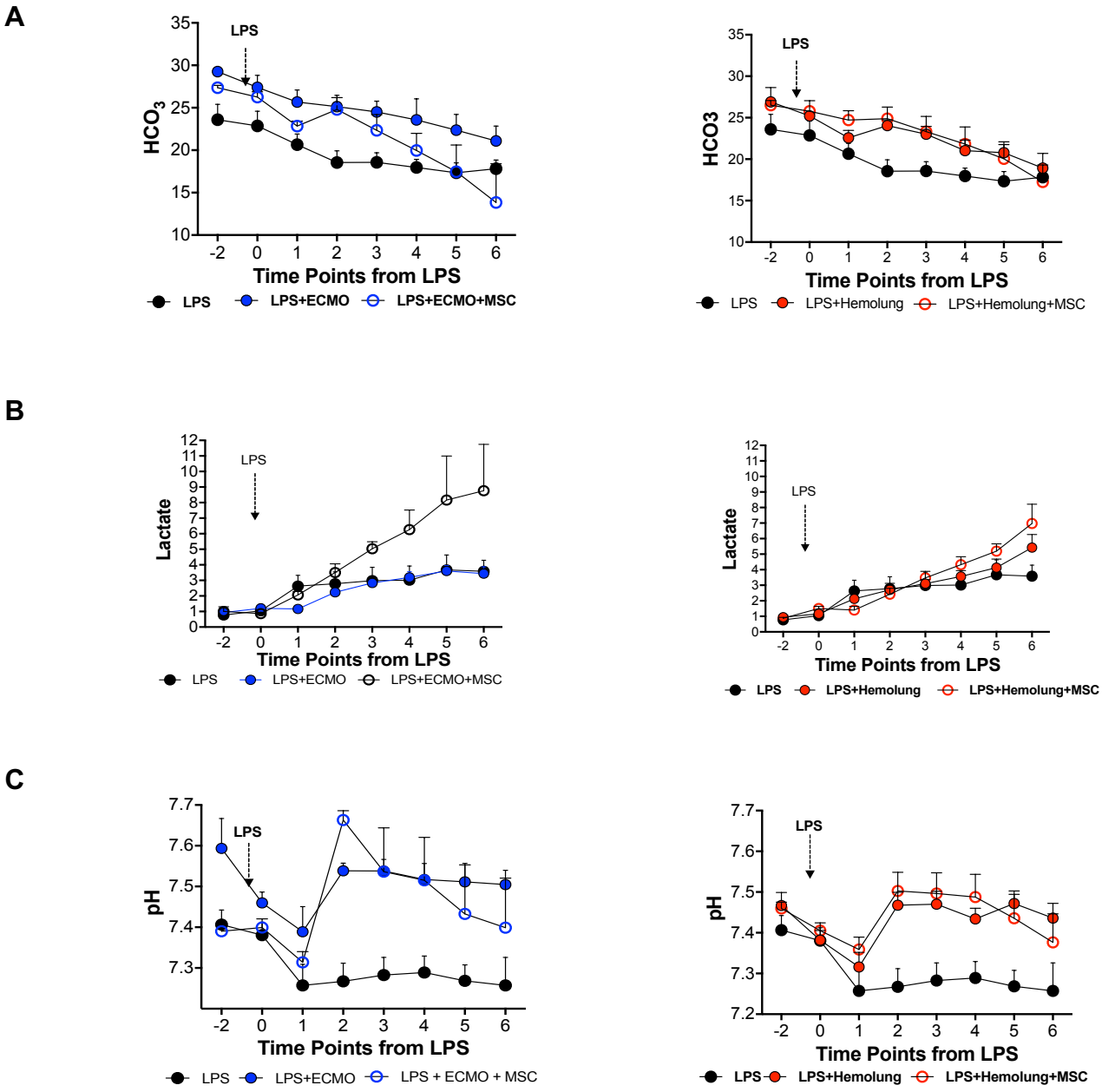

Figure 4. Changes in Cell Metabolism We observe metabolic acidosis in the ECMO-MSCs group in the different measurements; we observed a substantial increase in metabolic activity reflected by the increase in lactate and decrease in bicarbonate levels resulting on a decrease on the pH.

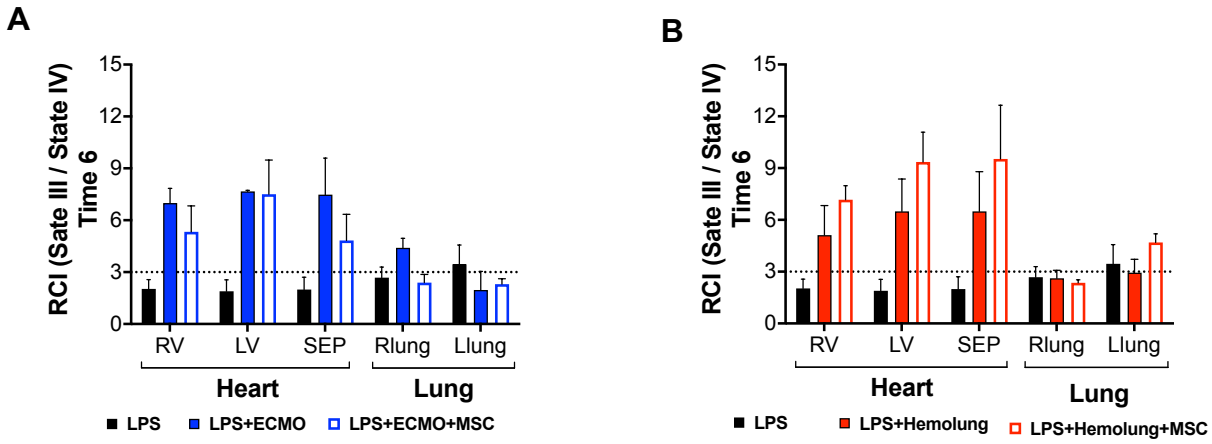

Figure 5. Mitochondrial Activy. To measure tissue injury, we measured oxygen consumption as a surrogate of mitochondrial activity. Our data suggest that devices and cell therapies provide heart protection. However, the only that protected the lungs and was the ALung+MSCs.

5. Conclusions:

The use of lung devices, combined with cell therapies, enabled reductions in the levels of injury in the acute setting of ARDS compared to Injured Controls. Also, in this case, we did not observe any of the interventions' harmful effects, suggesting that where all are safe for their use in the clinic. Further, MSCs did not demonstrate a substantial effect on mortality, development of ARDS, or end-organ injury severity than the devices alone and Injury Controls.

Summarizing, we observed that all the treatments improved outcomes or ARDS injury severity in this acute model. However, we were not able to demonstrate significant protection on the groups that received MSCs, as previously reported when MultiStem were. This work warrants further investigation of devices of different designs coupled with MultStem therapy.

6. Impact.

The development of new protocols to treat injured service members of the military forces can increase survival and reduce long-term complications. In this initial phase of the study, we confirmed that we could evaluate the protective effect of any intervention by using the proposed animal model.

As is presented in **Figure 1**, the use of ALung, a low invasive, low flow ECMO, can improve the conditions of blood oxygenation measured by the ratio PaO_2/FiO_2. We observed an improvement in respiratory parameters after 2 hours of respiratory support. This observation can have a tremendous impact because blood oxygenation may not be the primary indication of this system; the impact of pulmonary rest will reduce the time and severity of the lung injury.

This is the first study in which mitochondrial physiology is evaluated during ARDS; our preliminary data suggest that there is a decrease in mitochondrial function on specific areas of the heart during ARDS, which can result in a decrease of cardiac. Interestedly, only one group ALung+MSC protected mitochondria activity on the lung, suggesting that in this acute model, not aggressive interventions contribute to preserving the integrity of the lung.

7. Changes and Problems

As we described previously, some animals have severe complications during the surgical preparation of the animal, including the placement after an open chest of a central line, intracardiac cannulas in each ventricle, and respiratory support. Four experiments can not be included in the data because factors intrinsic in the animals like deficient oxygenation at the beginning of the experiment, are in the process to complete the statistical analysis of all the data.

Our main challenge, as we had mentioned before, was not to have the type of stem cell, the MultiStem, which had demonstrated, by other groups and us in pre-clinical assays to be effective to control ARDS in small and large animal models. That preliminary data was used to obtain an IND from the FDA to conduct a phase II clinical trial, which demonstrated safety and efficacy on patients with ARDS in the intensive care unit.

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8. Products

Manuscript under preparation,

9. Participants at the University of Pittsburgh

Personnel	Role
Mauricio Rojas MD Associate Professor Department of Medicine McGowan Institute of Regenerative Medicine University of Pittsburgh	PI
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Ergin Kocyildirim MD Research Assistant Professor Department of Cardiothoracic Surgery	Surgeon
Tomas Drabek MD Associate Professor Department of Anesthesiology	Anesthesiologist
Ron Poropatich MD Executive Director of the Center for Military Medicine Research, Professor of Medicine Division of Pulmonary, Allergy, and Critical Care Medicine University of Pittsburgh	Pulmonologist
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John Tedrow MD Assistant Professor Department of Medicine University of Pittsburgh	Pulmonologist
Nayra Cardenes PhD Instructor Department of Medicine University of Pittsburgh	Coordinator
Diana Alvarez MD Postdoctoral Fellow	Postdoc
Kentaro Nora Postdoctoral Fellow-Perfusionist	Perfusionist
Chandler Courfield Technician	Technician

10. Appendices

N/A

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

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1. INTRODUCTION:

This report serves as a final review of activities and progress made 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”.

By way of background, the current study was inspired by a previous study in Dr Batchinsky’s USAISR lab in San Antonio. That study pursued systemic administration of stem cells in the same model proposed in this study but without extracorporeal life support (ECLS). Similarly, previous work by Mauricio Rojas PhD, on utilization of stem cells in ex-vivo lung perfusion model served as background. Those studies have been crucial in understanding of how to conceptualize and execute the current award at both sites. First, in the Batchinsky lab we realized that administration of stem cells has a timed effect and repeat stem cell administration at high doses will be needed; second, a large body of work was carried out in our USAISR benchtop lab to characterize clonogenic capacity of MSCs but especially plastic adherence properties. The latter is due to concerns that administration of MSCs may be reduced in efficiency when ECLS circuit components are present in the blood. Third, we studied thrombogenic potential of MSCs ex-vivo to prepare for in-vivo administration of MSCs in this study. These steps permitted us to approach the current award with high confidence in a systematic validation study for concurrent use of mesenchymal stem cells (MSCs) and minimally invasive ECLS. Because at the time of the award the Batchinsky lab was actively performing experiments at the USAISR the experiments were planned to occur at USAISR, however significant institutional delays caused the place of performance to be relocated to Dr. Batchinsky’s second DOD-affiliated lab at Brooks City Base, San Antonio. Thus, the study was begun at ISR but completed at Brooks City Base.
Aside from the above delays noted at ISR, the lack of availability of mesenchymal stem cells for our study has been the central issue leading to delayed performance of experiments. Upon requesting cells from UPITT we received notification from Athersys, the partnering company of Dr. Rojas’s that they had been successful in a clinical trial in humans and that they were concerned about the potential negative effects of using human MSCs in swine in light of their FDA submission. We were also informed by Athersys of their inability to produce cells in quantities requested by Dr. Batchinsky for repeat administration.

To solve the problem, we began producing our own swine MSCs at ISR trying to bring the quantities up as needed for the study. Although much time and effort and supplies were used for this, we, in the end were unable to meet the needed MSC quantities on such a short notice. Dr. Batchinsky also reached out to commercial stem cell incubator producers (Terumo Medical, Golden, CO) with a request to test a stem cell incubator at ISR. This was carried out but after 3 months of verification work the incubator was returned to the company. The loan-related expenses were not charged to this grant but some materials and supplies related to stem cell storage and processing were. Although we were unable to solve our own cell production needs on a short notice, our effort did prove to be fruitful as this ex-vivo work laid the groundwork for development of a new in-

house ISR capabilities: a true example of benefit via leveraging an extramural funding to establish an intramural research area. We created a library and laboratory for stem cell production within the Multiorgan Support Technology Task Area (TA) at the ISR and characterized thrombogenic properties of the stem cells in anticipation of in-vivo use in the study. These accomplishments led to significant cost savings and productivity enhancement to the overall DOD efforts with regenerative medicine which were led by Dr. Batchinsky since inception of this program.

The loss of commercial MSC provider without designated replacement led to significant delay, and forced Dr. Batchinsky to expend great effort to secure the required cells for the completion of this work. Once a new MSC source was identified locally in San Antonio (BioBridge Global, San Antonio, Texas) we purchased cells for the study (~February 2019), the remaining work on this award was completed at BCB within 7 months.

2. ACCOMPLISHMENTS:

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

Major Task 2 and its milestones have been completed – see SOW.

Major Task 3 “To determine the safest combination of cell therapy and ECMO, by evaluating the two models of ECMO with MAPCs”.

For Dr. Batchinsky’s portion of Major Task 3, he was to conduct three experimental groups in swine: 1) ARDS induced by smoke inhalation and 40% TBSA burn (planned n=10), 9 completed (2 at ISR and 7 at BCB) one animal was excluded due to broken furnace and technical no -go/inability to generate smoke inhalation injury; 2) ARDS induced by smoke inhalation and 40% TBSA burn treated with low-flow ECLS only (planned n=15), 12 completed (5 at ISR, 7 at BCB) 3 animals were transferred to group 3; 3) and ARDS induced by smoke inhalation and 40% TBSA burn treated with ECLS + MAPCs (planned n=15), 13 completed (all at BCB with 3 transferred from group 2 (see explanation below)).

What was accomplished under these goals?

All animals and experiments were completed with some animals reclassified from group 2 to group 3 because of the following 2 factors.

1) In the MSC+ECLS group, the first 5 animals experienced symptoms of anaphylactic shock following repeat administration of stem cells. Additionally, complications including renal and kidney failure were observed. In this subset of 5, the survival time was 39 ± 9 hours, and time to ARDS was 8 ± 5 hours (Table 1 shows survival time and time to ARDS for the other animals for comparison). Additionally, 2 of the 5 animals had acute kidney injury (according to the Kidney Disease Improving Global Outcomes [KDIGO] criteria) with mean time to onset of 2 hrs post-injury. All 5 required emergency fluid administration and pressors (epinephrine) to support circulation and cardiovascular function. Following repeat

observations of these complications, we consulted with the stem cell vendor, BioBridge Global. After additional studies, it was determined that there was an elevated level of human endotoxin in the cell media, requiring us to exclude these 5 animals. The vendor modified their cell preparation protocol to address this issue for the remainder of the ECLS+MSC group.

2) We moved 3 animals into the MSC treatment group from the ECLS only group in which the lack of ECLS benefit was already clinically apparent.

Table 1 summarizes the animal demographics, injury metrics, and survival times for the 3 groups.

		Mean ± SEM				
Group	**Completed *n***	**Weight kg**	**Smoke Breaths Received**	**Max CoHb%**	**Time to ARDS (h)**	**Survival Time (h)**
1 (Injured Control)	9	52.0 ± 1.8	43 ± 4	79 ± 1	13.3 ± 5.3	72.0 ± 0.0
2 (ECLS Only)	12	50.8 ± 2.2	38 ± 4	77 ± 2	21.3 ± 6.7	56.2 ± 6.0
3 (ECLS + MSC)	13	51.7 ± 2.5	50 ± 1	77 ± 2	14.1 ± 6.4	57.2 ± 6.8

Table 1. Summary demographics of experimental groups.

In groups 2 and 3, VV-ECLS therapy was initiated post-injury, and performed via a 15.5 Fr. dual-lumen catheter inserted by modified Seldinger technique into the animal's right jugular vein. Using air-free, wet-to-wet connections, this catheter was connected to the Hemolung device (ALung Technologies, Pittsburgh, PA), and flow was started. In all animals, the Hemolung pump was set to 1400 RPM, with sweep gas set at 10 Lpm, FiO_2 1.0.

In animals that received MSC administration in addition to ECLS therapy, the MSCs were administered immediately following ECLS initiation, and at 24- and 48hr. post-injury. The mean concentration of administered cells was $5.87 \pm 0.18 \times 10^6$ cells/mL.

Cells were produced by BioBridge Global (San Antonio, TX), and delivered in ready-to-administer IV bags of 50 mL total volume each, and stored at 4 °C until use. Prior to use, the cells were allowed to come to room temperature over a period of 30 minutes while rocking on a sample tube rocker. Cells were manually aspirated from the bag via 60 mL syringe, and the bag was rinsed with 30 mL heparinized saline to ensure all cells were collected. The cells were administered over a period of 2 minutes directly into the blood return line of the ECLS system into a port inserted 10 cm before the catheter in the blood return line. Following cell administration, the port was flushed through with 30 mL of heparinized saline.

Mean±SEM MSC doses received are shown in Figure 1:

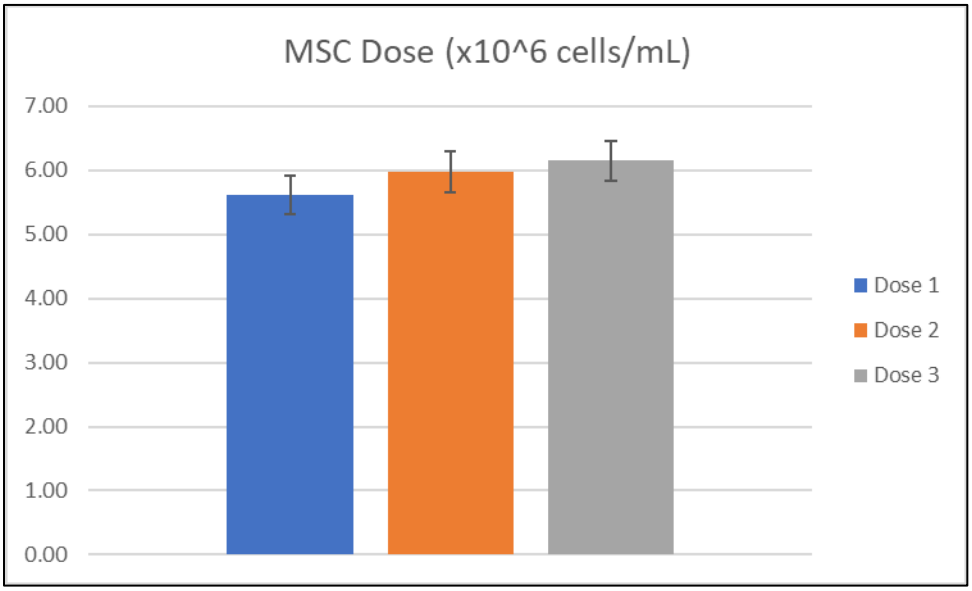

Figure 1. Quantity of cells administered per dose, data mean±SEM.

Quantification of cells administered per dose, per kg of animal body weight, is shown in Figure 2:

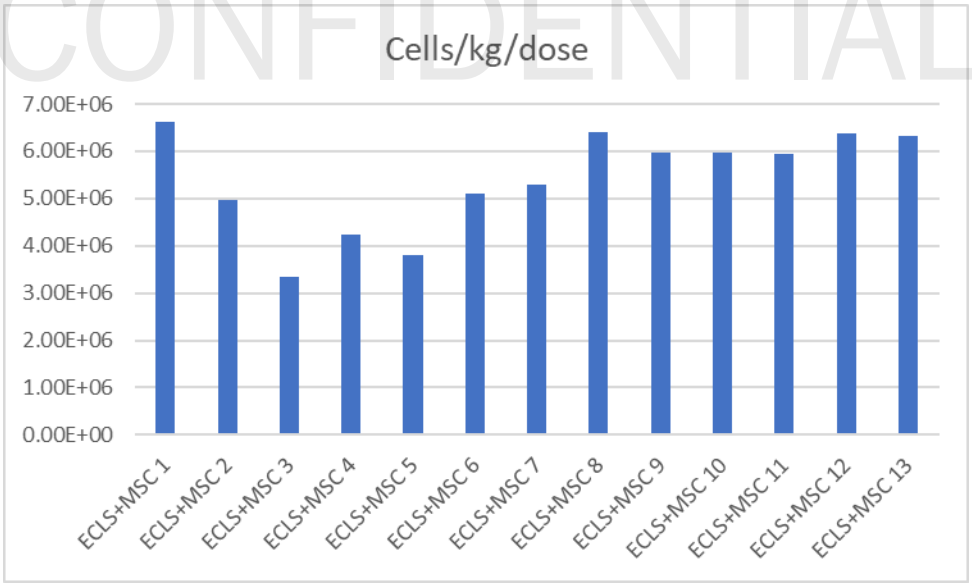

Figure 2. MSC administered per kg animal weight, per dose.

Mortality at 72 hours post-injury was 0% in the injured control group (9/9 surviving to 72 hours), 50% in the ECLS only group (6/12 surviving to 72 hours), and 31% in the ECLS+MSC group (9/13 surviving to 72 hours). No significant difference between groups in demographic information was detected. No difference in survival time between groups was detected (Figure 3).

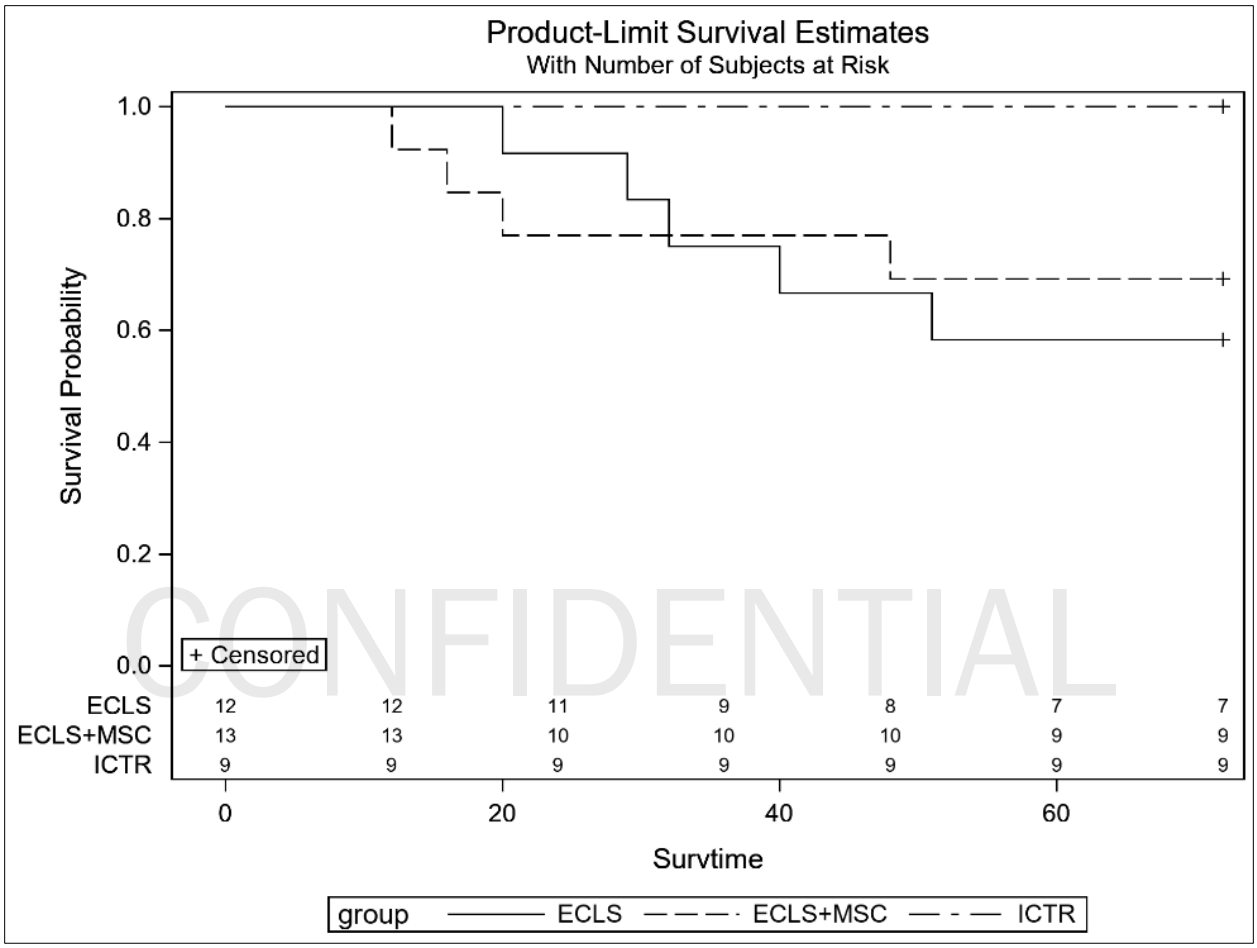

Figure 3. Survival probability curve. No statistical difference in survival times identified, and no significant difference in incidence of mortality detected.

Analysis of probability of ARDS development (defined as PFR<300) is shown in Figure 4. No between group difference in time to ARDS was detected.

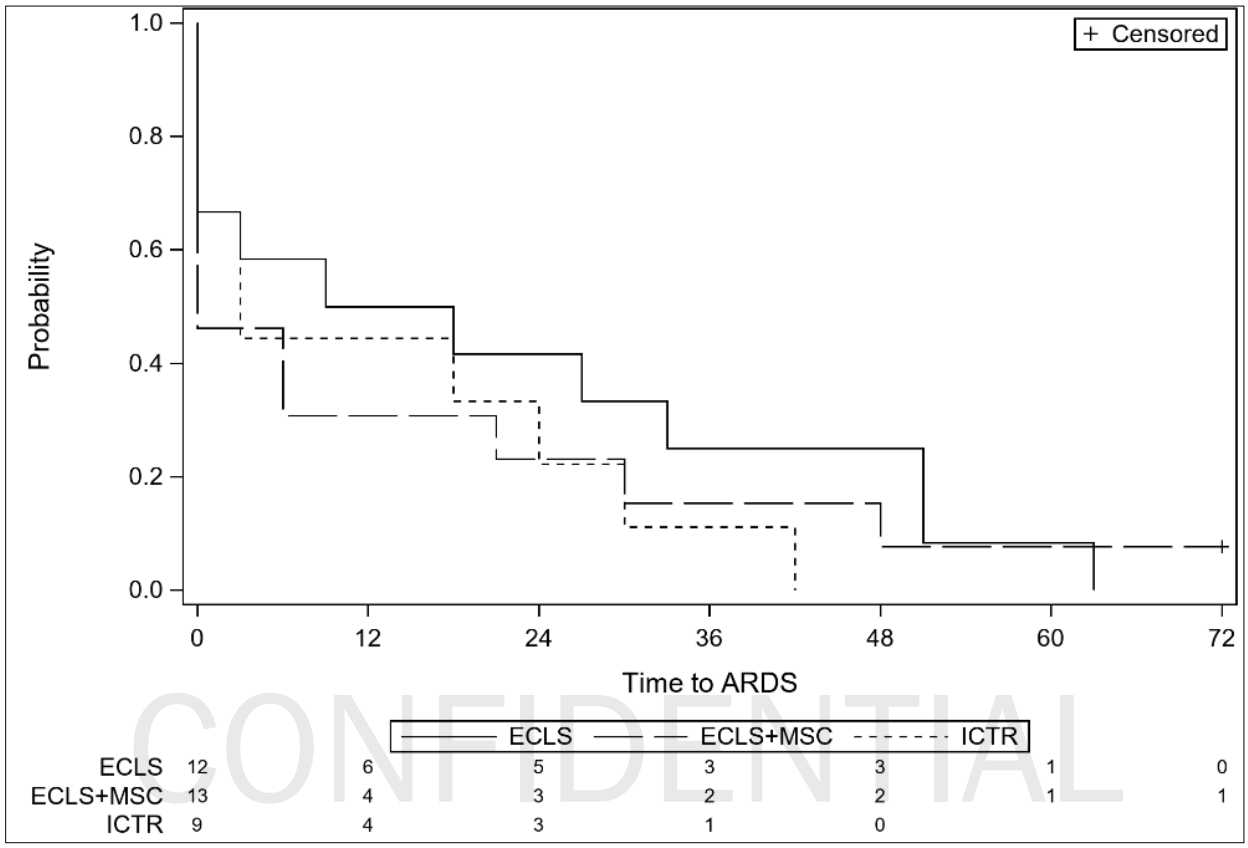

Figure 4. Probability of ARDS development. No between-group significance identified.

The ARDS severity index PaO2 to FiO2 Ratio (PFR) is shown below, with no significant difference between groups except a rebound of PFR at 24 and 48 hours in the ECLS group (Figure 5).

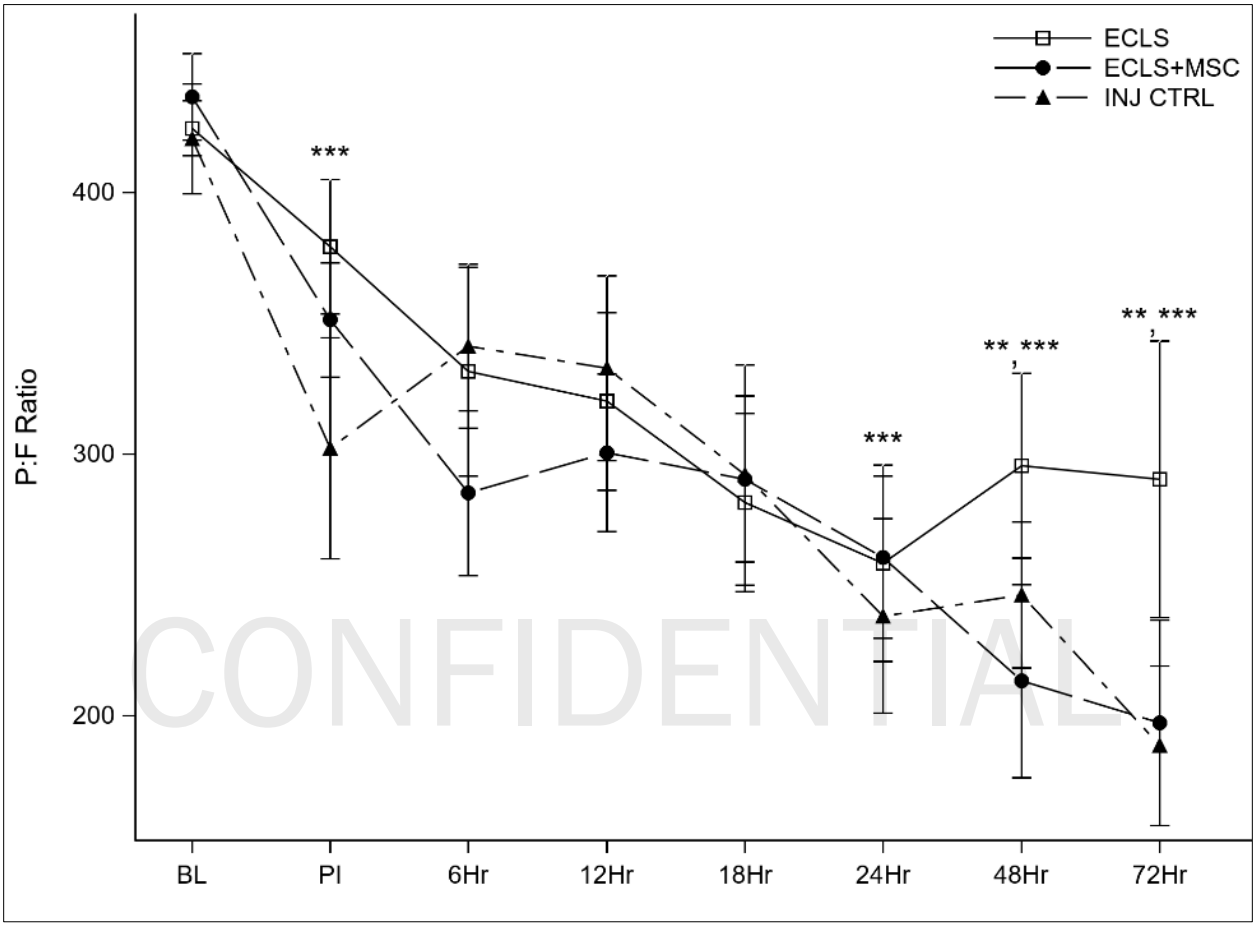

*Figure 5. PaO2 to FiO2 Ratio (PFR). *ECLS difference from baseline. **ECLS+MSC difference from baseline. ***Injured control difference from baseline. All statistics p<0.05*

We also did not identify between group differences when analyzing time to development of different stages/severities of ARDS (Figure 6). Mild ARDS is defined as 200≤PFR<300, moderate ARDS defined as 100≤PFR<200, severe ARDS defined as PFR<100.

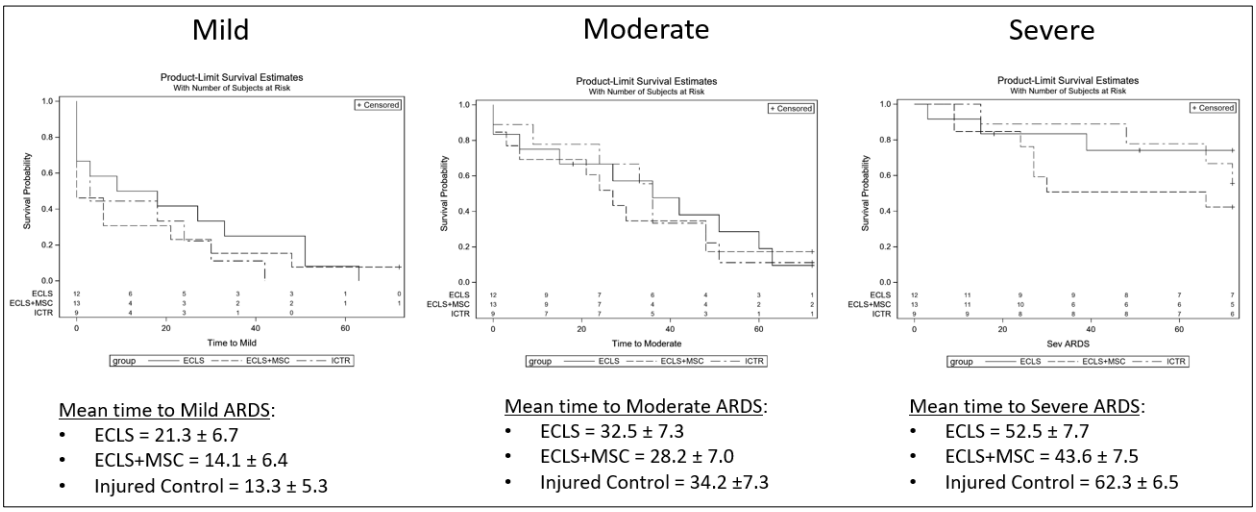

Figure 6. By-severity development of ARDS analyzed by experimental group. No between-group differences identified.

Figure 7 shows the percentage of each experimental group developing each severity category of ARDS over the course of the study:

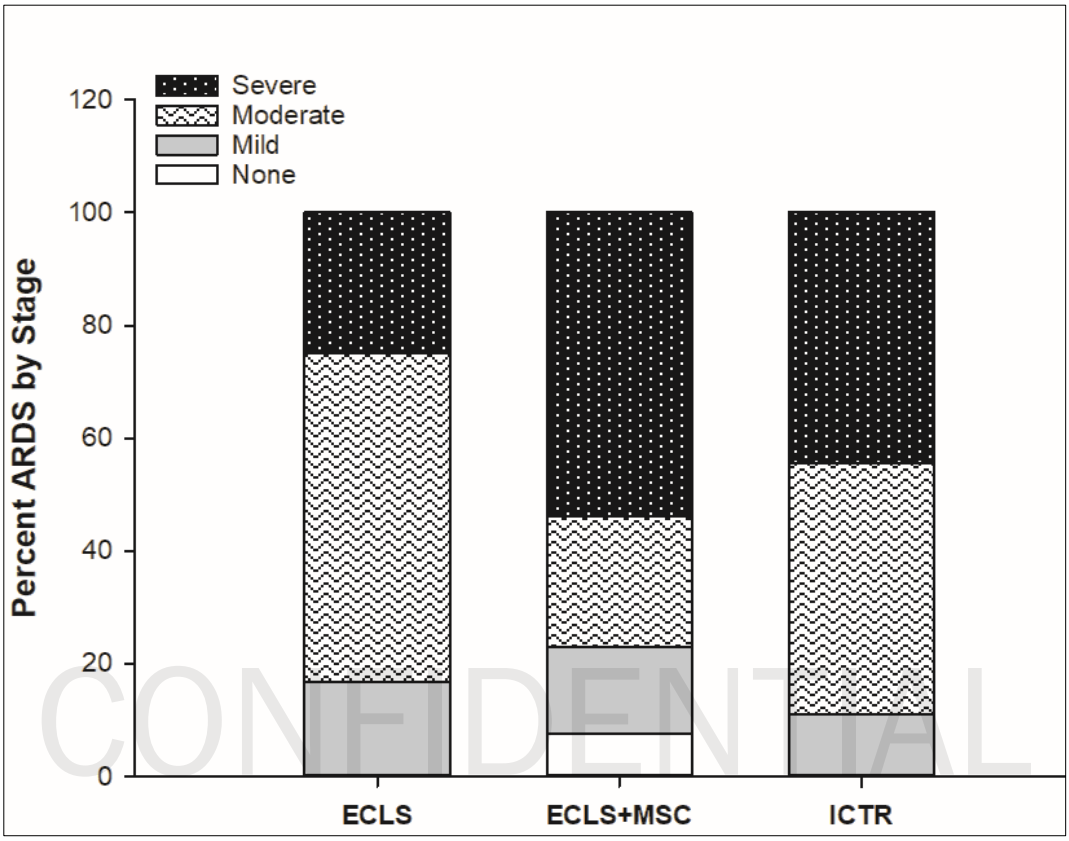

Figure 7. Percent of the study population by group that reached each of stage of ARDS. Each animal is classified by the most severe stage of ARDS that they reached (regardless of survival time).

Hemodynamic variables and vitals are shown in Table 2, below, as mean ± SEM:

Variable:	**Group:**	**Time Point:**				
		BL	**PI**	**24h**	**48h**	**72h**
HR (bpm)	ECLS	93 ± 5	112 ± 11	99 ± 7	103 ± 6	103 ± 5
	ECLS + MSC	107 ± 6	151 ± 9*	96 ± 6	105 ± 5	114 ± 8
	Injured Control	102 ± 5	109 ± 9	109 ± 7	102 ± 8	104 ± 6
ABP/S (mmHg)	ECLS	115 ± 3**†,§**	122 ± 6	115 ± 4†	113 ± 5	109 ± 5
	ECLS + MSC	102 ± 4	119 ± 6*	94 ± 3	106 ± 4	104 ± 4
	Injured Control	99 ± 4	123 ± 10*	101 ± 5	107 ± 4	107 ± 3
ABP/D (mmHg)	ECLS	76 ± 4†	83 ± 6	71 ± 6†	62 ± 4	55 ± 6*
	ECLS + MSC	59 ± 3	76 ± 5*	55 ± 3	56 ± 4	54 ± 3
	Injured Control	65 ± 5	87 ± 8*	60 ± 5	58 ± 4	58 ± 3
MAP (mmHg)	ECLS	94 ± 3 **†,§**	98 ± 5	90 ± 5 †	84 ± 4	79 ± 5
	ECLS + MSC	77 ± 3	93 ± 5*	73 ± 3	78 ± 4	77 ± 4
	Injured Control	79 ± 4	103 ± 8*	78 ± 5	79 ± 4	81 ± 2
SpO_2 (%)	ECLS	97 ± 1	96 ± 1	94 ± 2	95 ± 2	98 ± 1
	ECLS + MSC	97 ± 0	97 ± 1	96 ± 1	95 ± 3	95 ± 2
	Injured Control	98 ± 1	96 ± 1	95 ± 1	95 ± 2	97 ± 1
Urine Output (mL/hr)	ECLS	142 ± 46	32 ± 5*	67 ± 16	96 ± 10	82 ± 13
	ECLS + MSC	96 ± 32	49 ± 13	104 ± 14	115 ± 20	98 ± 22
	Injured Control	144 ± 47	43 ± 10*	114 ± 28	137 ± 24	93 ± 25
Temperature (˚C)	ECLS	41.2 ± 5.0	37.8 ± 0.4	36.9 ± 0.5 **§**	37.8 ± 0.2	37.6 ± 0.2 **§**
	ECLS + MSC	36.4 ± 0.2	38.1 ± 0.4*	37.0 ± 0.4 **‡**	37.3 ± 0.2* **‡**	37.5 ± 0.2* **‡**
	Injured Control	36.4 ± 0.3	38.0 ± 0.3*	38.8 ± 0.3*	38.3 ± 0.2*	38.4 ± 0.2*

*Table 2. Hemodynamic variables by group. BL; Baseline, PI; post-injury. *significant change from baseline value; † ECLS versus ECLS+MSC; § ECLS versus Injured Control; ‡ ECLS+MSC versus Injured Control, p<0.05.*

Lactate levels (Figure 8) were elevated in every group at post-injury; however, there were no between group differences.

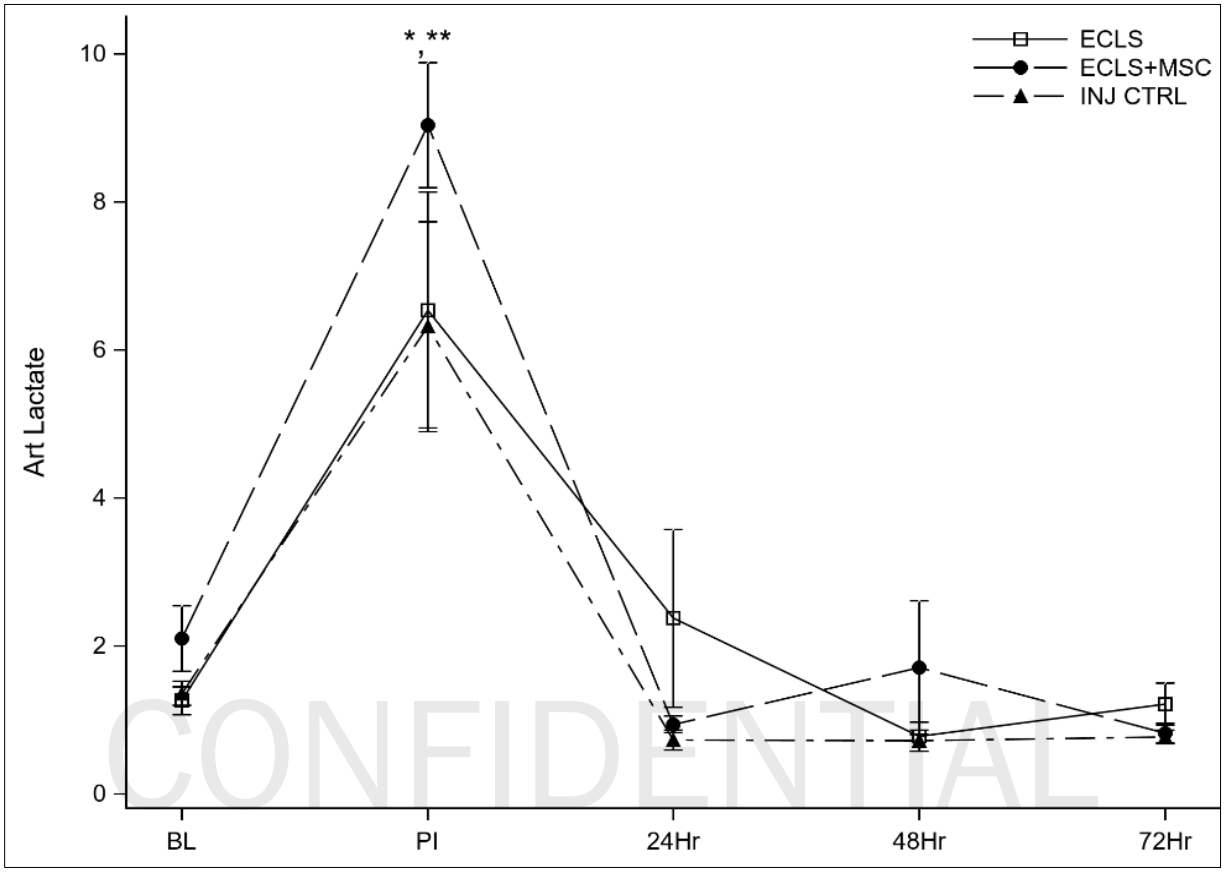

*Figure 8. Arterial lactate. *ECLS difference from baseline. **ECLS+MSC difference from baseline. ***Injured control difference from baseline. All statistics p<0.05*

Respiration rate (Figure 9) was significantly higher in the Injured Control Group compared to both the ECLS group and the ECLS+MSC group. At 48 hours, respiration rate was significantly lower in ECLS versus ECLS+MSC.

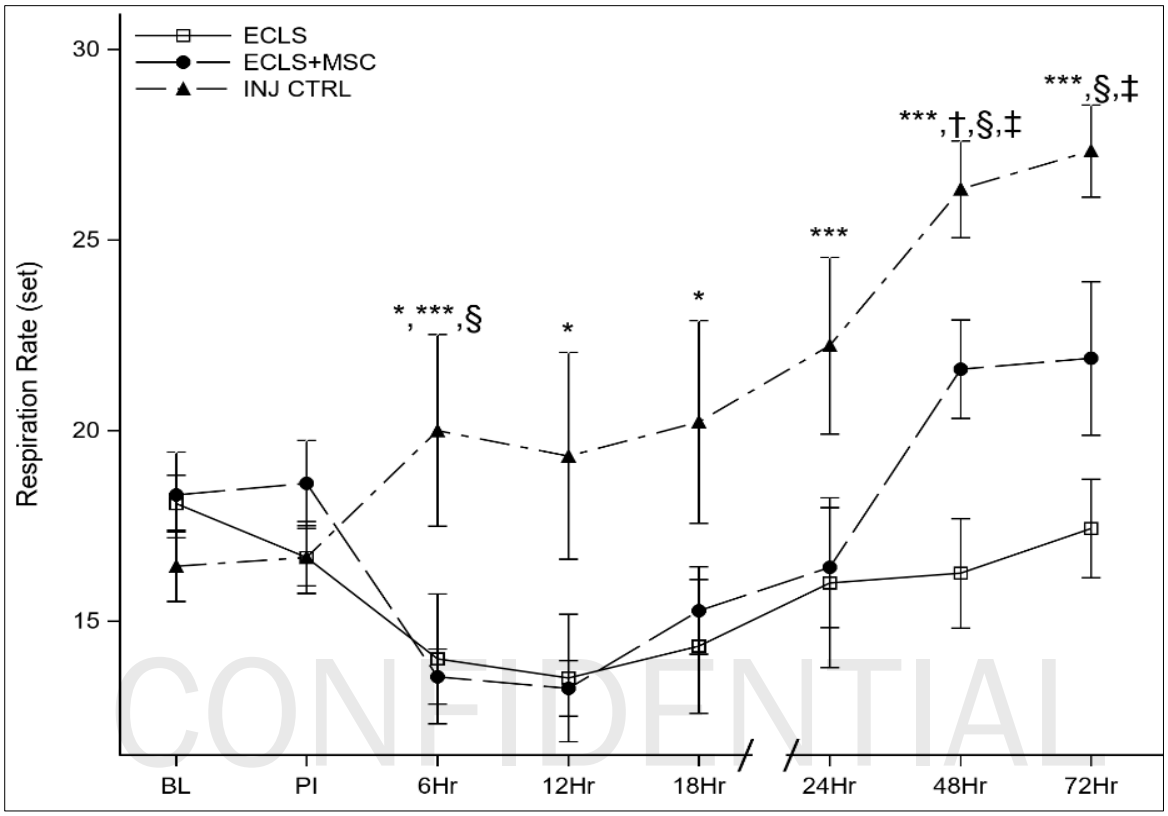

*Figure 9. Respiration rate. *ECLS difference from baseline. **ECLS+MSC difference from baseline. ***Injured control difference from baseline. † ECLS versus ECLS+MSC; § ECLS versus Injured Control; ‡ ECLS+MSC versus Injured Control. All statistics p<0.05*

Use of Hemolung in the ECLS and ECLS+MSC group enabled a reduction in minute volume (Figure 10) compared to Injured Controls that persisted to 24 hours in both groups. From 48-72, minute volume was significantly reduced in the ECLS group versus Injured Control but was no longer reduced in ECLS+MSC.

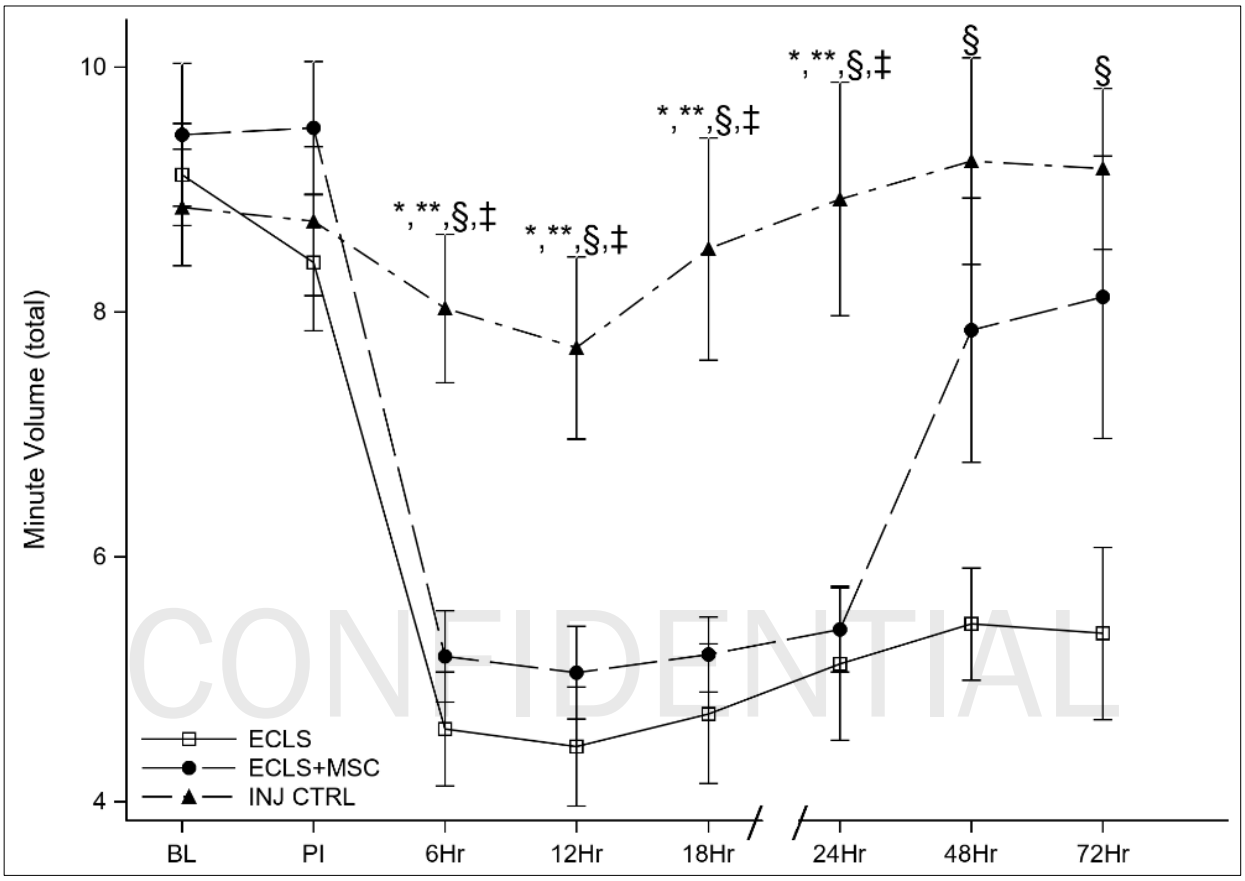

*Figure 10. Minute Volume. *ECLS difference from baseline. **ECLS+MSC difference from baseline. ***Injured control difference from baseline. † ECLS versus ECLS+MSC; § ECLS versus Injured Control; ‡ ECLS+MSC versus Injured Control. All statistics p<0.05*

There were no between-group differences in FiO2 (Figure 11) or peak inspiratory pressure (PIP) (Figure 12). In all groups, FiO2 and PIP increased with time after injury.

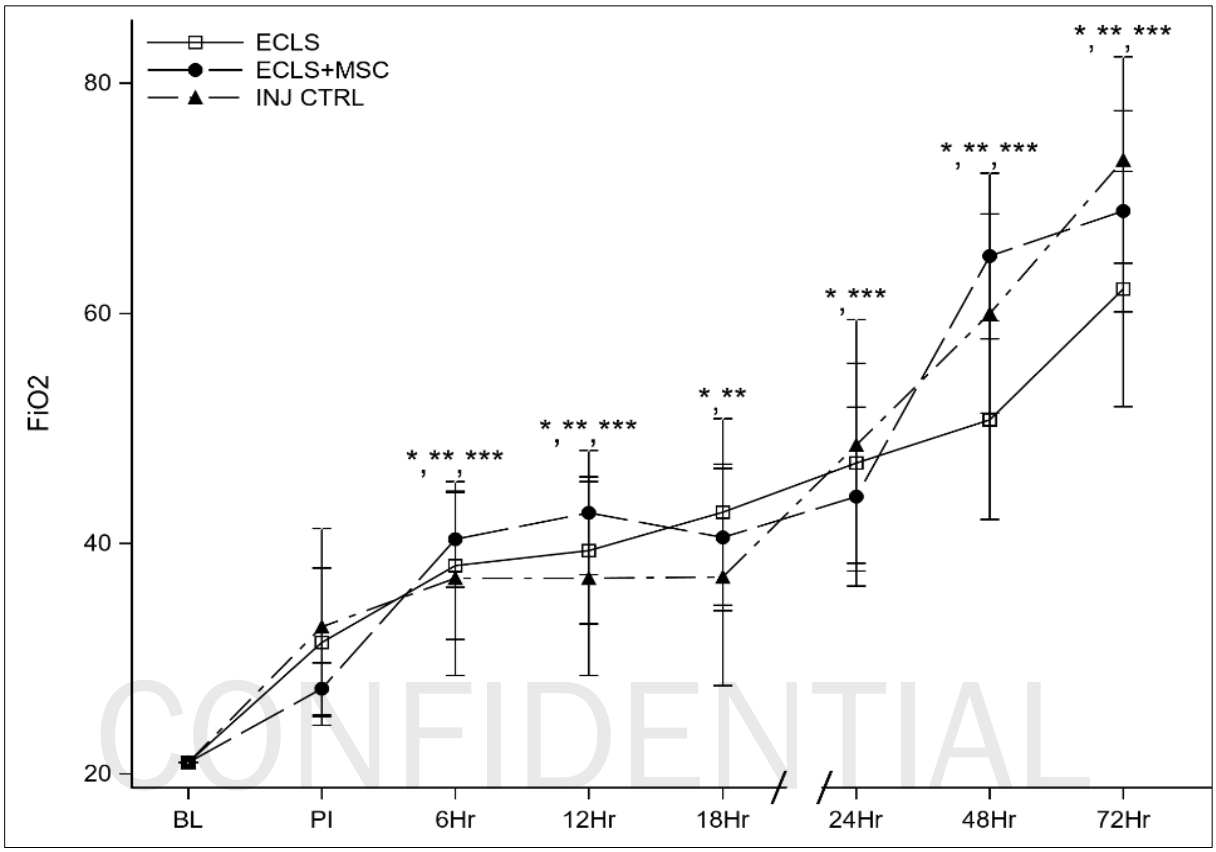

*Figure 11. Fraction of inspired oxygen (FiO). *ECLS difference from baseline. **ECLS+MSC difference from baseline. ***Injured control difference from baseline. All statistics p<0.05*

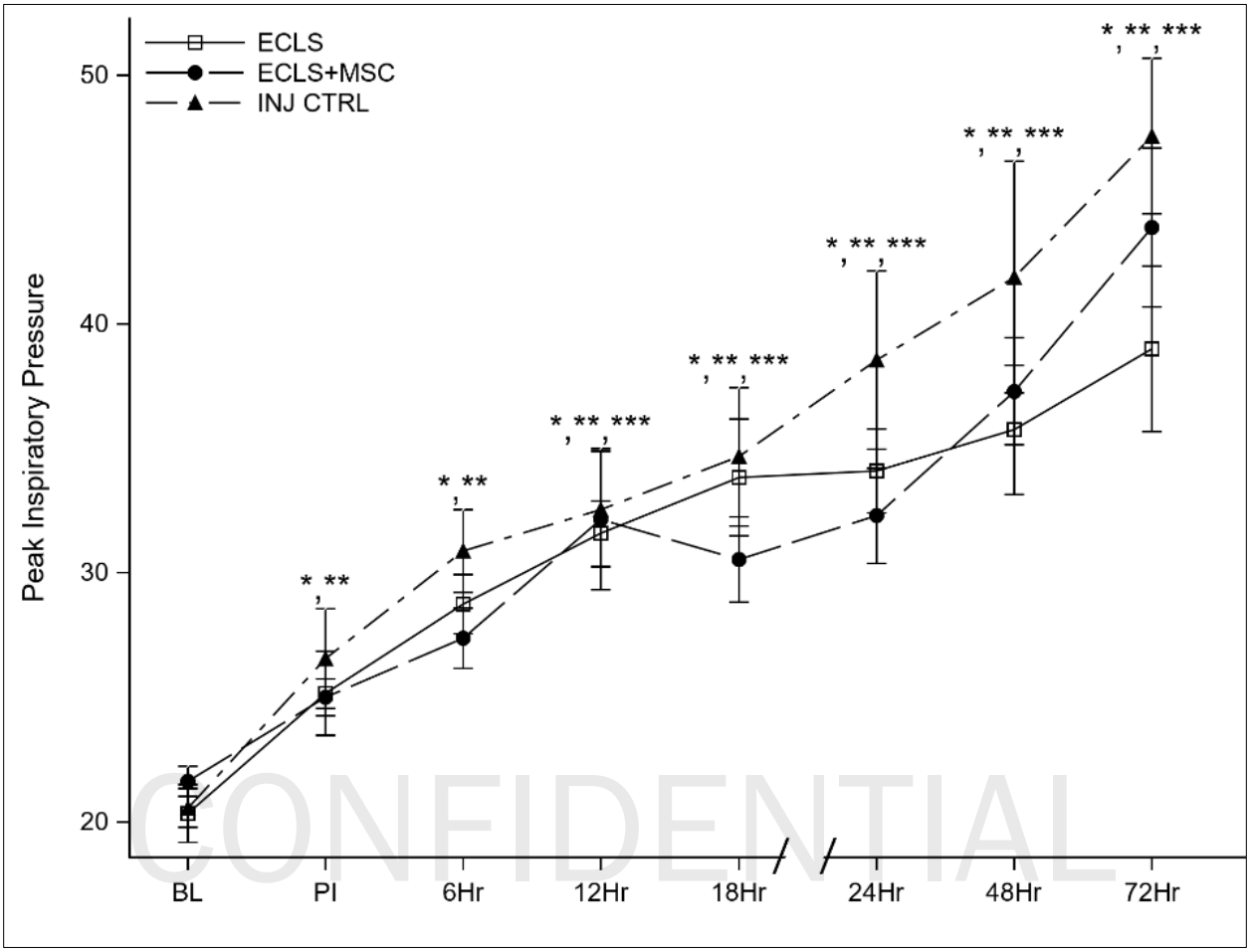

*Figure 12. Peak Inspiratory Pressure. *ECLS difference from baseline. **ECLS+MSC difference from baseline. ***Injured control difference from baseline. All statistics p<0.05*

Table 3, below, lists other measured respiratory variables recorded throughout the study, broken down by group as mean ± SEM:

Variable:	Group:	Time Point:				
		BL	PI	24h	48h	72h
OI (%)	ECLS	2.1 ± 0.1	2.9 ± 0.4	13.7 ± 5.1*	8.7 ± 2.9*	8.4 ± 1.8*
	ECLS + MSC	2.3 ± 0.2	3.3 ± 0.4	9.6 ± 2.1*	19.8 ± 7.6*	23.1 ± 6.5*
	Injured Control	2.3 ± 0.1	5.4 ± 1.5	13.1 ± 5.1*	10.0 ± 2.1*	14.1 ± 2.0*
SFR	ECLS	464 ± 2	373 ± 34	280 ± 44	229 ± 40*	182 ± 26*
	ECLS + MSC	463 ± 2	376 ± 24	271 ± 36	164 ± 18*	151 ± 24*
	Injured Control	465 ± 3	372 ± 41	297 ± 46*	197 ± 25*	158 ± 21*
FiO2 (%)	ECLS	21 ± 0	31 ± 6	47 ± 9*	51 ± 9*	62 ± 10*
	ECLS + MSC	21 ± 0	27 ± 2	44 ± 8	65 ± 7*	69 ± 9*
	Injured Control	21 ± 0	33 ± 9	49 ± 11*	60 ± 9*	73 ± 9*
Compliance (mL/cm H_2O)	ECLS	44 ± 3	33 ± 2	19 ± 2*	19 ± 2*	16 ± 2*
	ECLS + MSC	37 ± 2	31 ± 2*	18 ± 2*	15 ± 2*	14 ± 2*
	Injured Control	42 ± 2	32 ± 3*	19 ± 2*	15 ± 1*	13 ± 1*
Resistance (cm H_20/L/s)	ECLS	6 ± 0	9 ± 1	11 ± 1	16 ± 2*	17 ± 4*
	ECLS + MSC	6 ± 0	7 ± 0	10 ± 1*	12 ± 2*	14 ± 1*
	Injured Control	6 ± 0	9 ± 1*	13 ± 2*	16 ± 3*	20 ± 3*
VCO2 (mL/min)	ECLS	270 ± 21	215 ± 19	112 ± 18*§	150 ± 20	139 ± 32
	ECLS + MSC	287 ± 19	265 ± 15	138 ± 9*‡	172 ± 17	176 ± 17
	Injured Control	259 ± 27	237 ± 25	214 ± 18*	200 ± 16*	205 ± 20
etCO2 (mmHg)	ECLS	42 ± 1	36 ± 1	38 ± 3	41 ± 2	43 ± 4
	ECLS + MSC	39 ± 1	38 ± 1	38 ± 1	37 ± 4	36 ± 3
	Injured Control	41 ± 1	37 ± 2	39 ± 3	41 ± 4	42 ± 4

*Table 3. Respiratory variables recorded by group. *significant change from baseline value; † ECLS versus ECLS+MSC; § ECLS versus Injured Control; ‡ ECLS+MSC versus Injured Control, p<0.05.*

The reduction in minute volume observed in the Hemolung groups (ECLS and ECLS+MSC) is attributed to removal of CO2 by the Hemolung device. Partial Pressure of CO_2 in arterial blood (Figure 13) was elevated in the Injured Controls, even with the elevated mechanical ventilatory support.

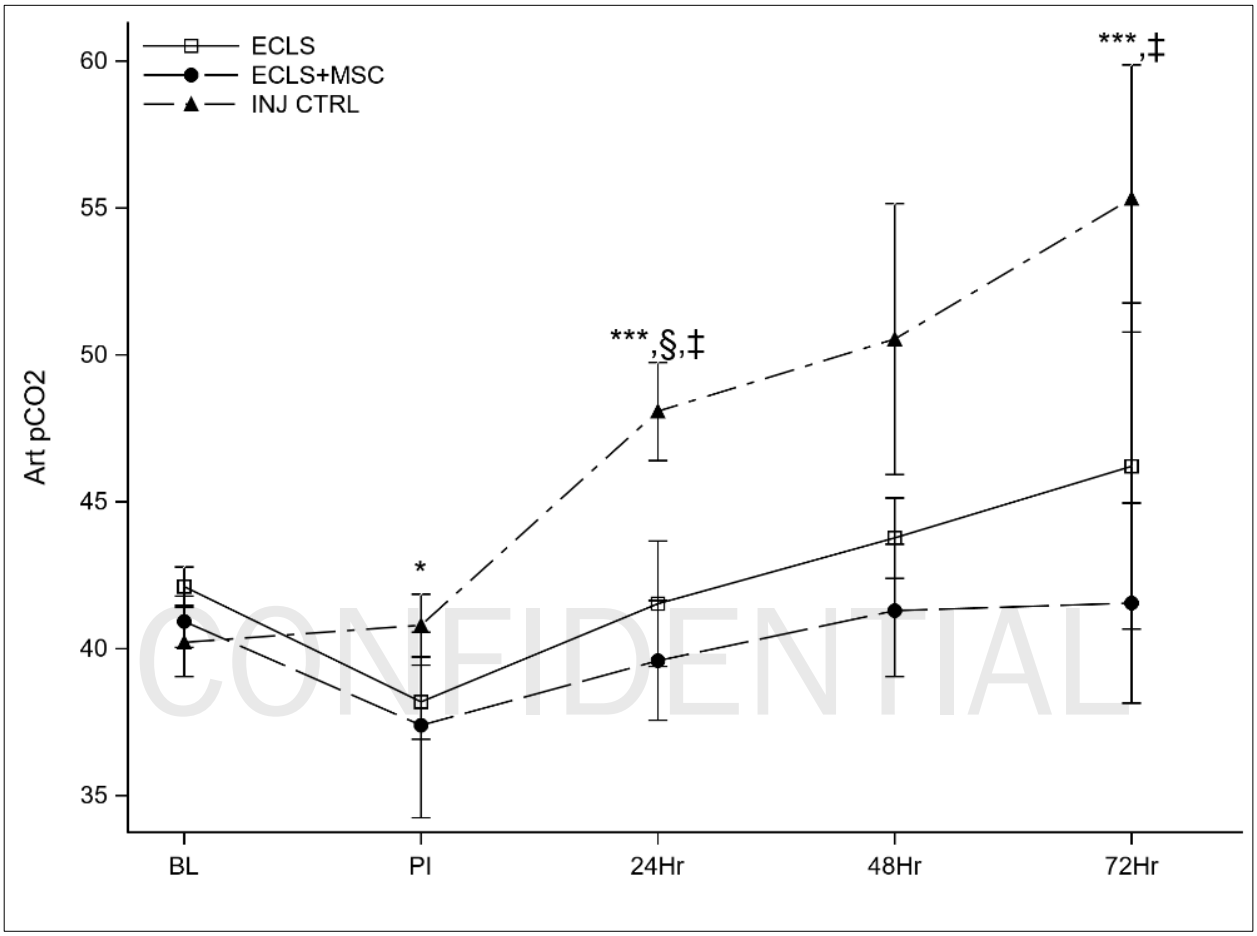

*Figure 13. Partial Pressure of CO_2 in arterial blood. *ECLS difference from baseline. **ECLS+MSC difference from baseline. ***Injured control difference from baseline. † ECLS versus ECLS+MSC; § ECLS versus Injured Control; ‡ ECLS+MSC versus Injured Control. All statistics p<0.05*

Blood gas samples collected immediately before the Hemolung membrane (pre-membrane) and after blood passage through the membrane prior to return to systemic circulation (post-membrane) demonstrate a significant reduction in PCO2 in both the ECLS and ECLS+MSC groups (Figure 14). PCO2 in pre-membrane blood versus post-membrane blood was significant at all time points (significance markers not displayed on figure).

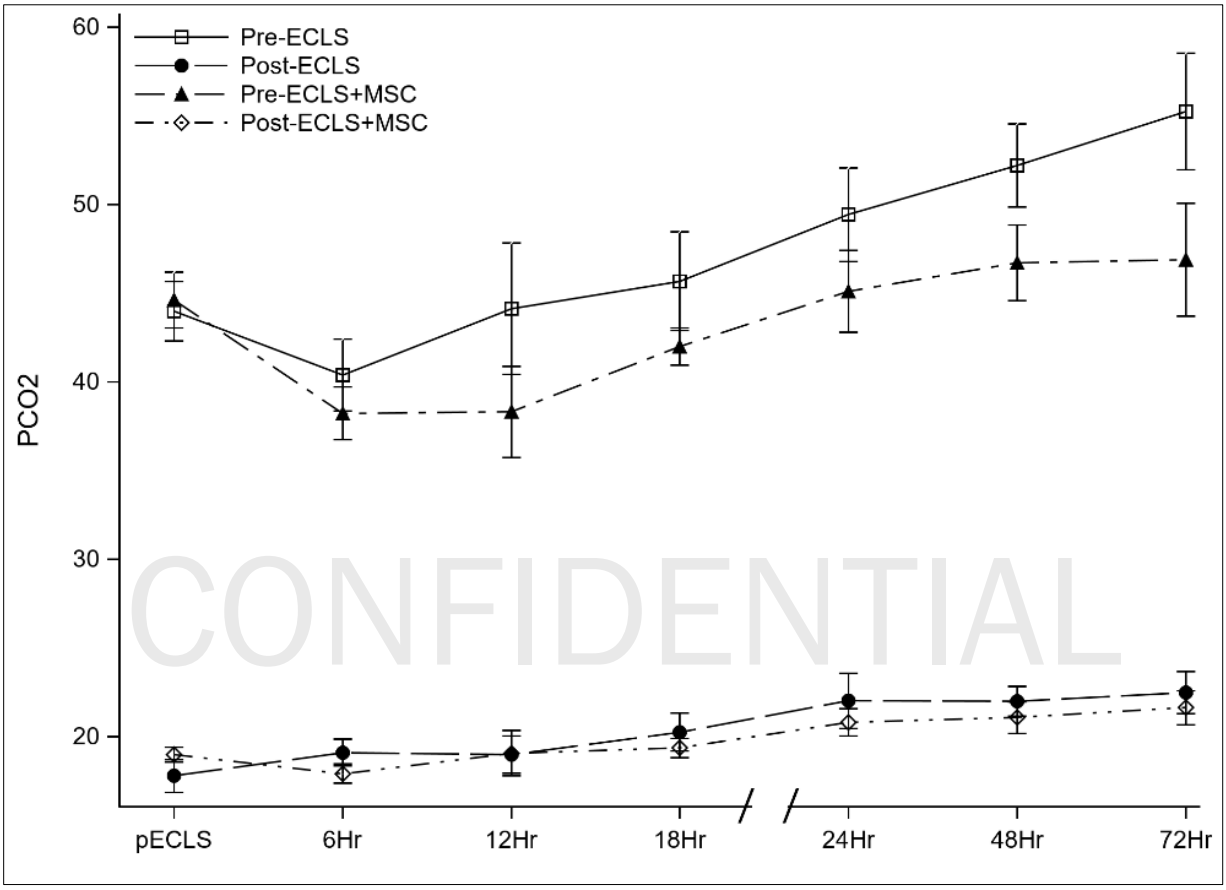

Figure 14. CO_2 in pre- and post-membrane blood gas samples (solid line for ECLS group, dashed line for ECLS+MSC group). At all time points, the difference between Pre- and Post- samples within groups is significantly different (significance markers not shown). All statistics $p<0.05$.

The percent reduction in pCO_2 across the membrane (% CO_2 removed) in the ECLS and ECLS+MSC groups is shown in Figure 15. % CO_2 removed was consistent throughout (no change within groups over time) and was not different between groups.

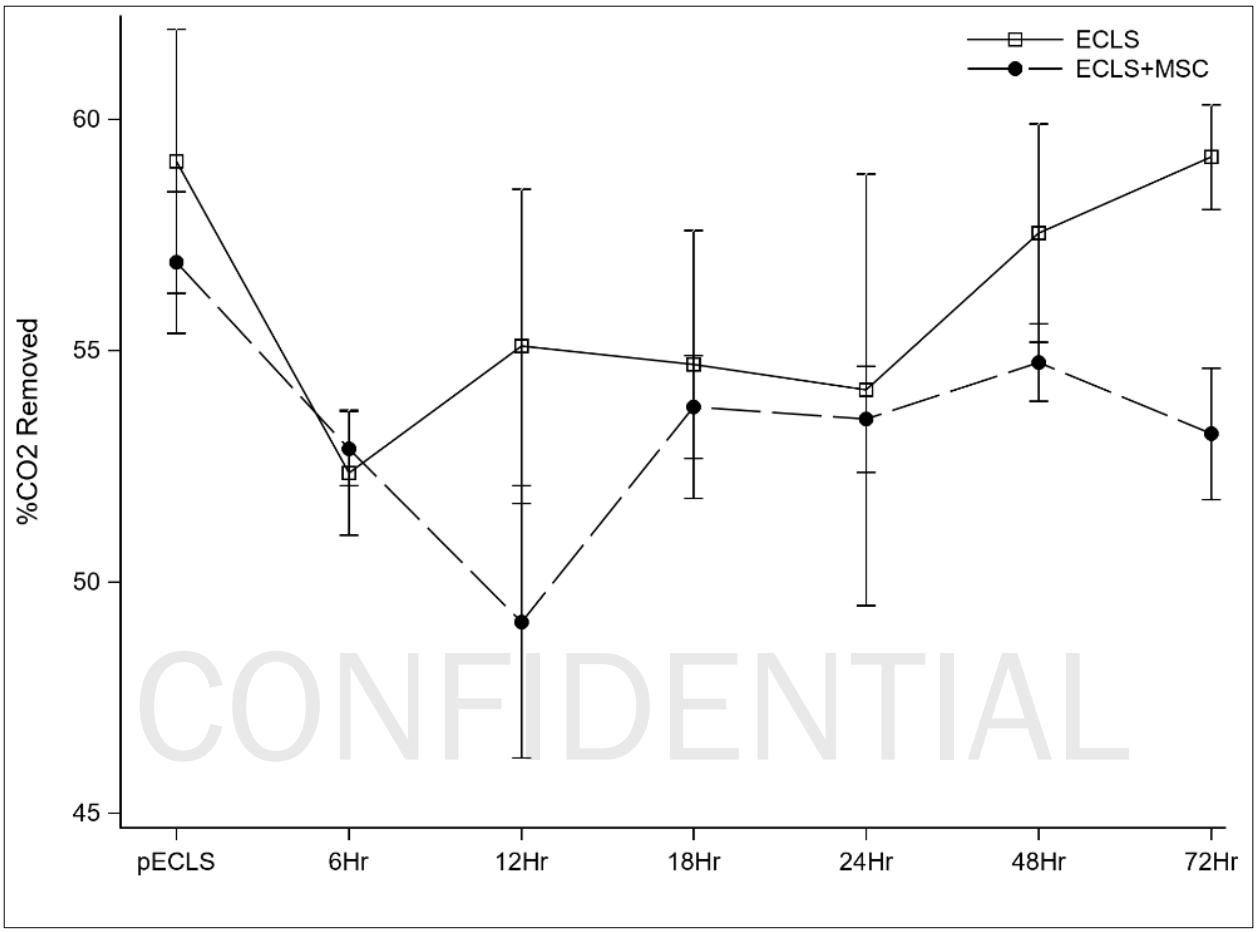

Figure 15. Percent reduction in pCO2 from pre-membrane blood gas samples to post-membrane blood gas samples. No statistically significant differences within or between groups was detected. p<0.05.

Table 4, below, shows additional arterial blood gas and chemistry variables recorded throughout the study, broken down by group as mean ± SEM:

Variable:	Group:	Time Point:				
		BL	PI	24h	48h	72h
pH	ECLS	7.49 ± 0.01	7.43 ± 0.03	7.39 ± 0.04	7.44 ± 0.01	7.45 ± 0.03
	ECLS + MSC	7.51 ± 0.01	7.38 ± 0.02*	7.42 ± 0.02	7.40 ± 0.05	7.43 ± 0.04
	Injured Control	7.51 ± 0.01	7.41 ± 0.03*	7.35 ± 0.04*	7.40 ± 0.03*	7.39 ± 0.01*
pO2 (mmHg)	ECLS	89 ± 2	122 ± 32	97 ± 15	151 ± 40	184 ± 54*
	ECLS + MSC	92 ± 3	92 ± 5	97 ± 7	125 ± 23	110 ± 11
	Injured Control	88 ± 4	76 ± 4	86 ± 5	141 ± 22	136 ± 32
Base Excess (mmol/L)	ECLS	9.1 ± 1.0	1.8 ± 2.3	0.6 ± 2.2*	5.2 ± 0.9	7.3 ± 2.1
	ECLS + MSC	9.6 ± 1.1	-0.9 ± 1.4*	1.2 ± 1.4*	1.6 ± 2.7	3.0 ± 1.5
	Injured Control	9.7 ± 1.4	3.3 ± 2.2*	1.8 ± 2.7*	6.1 ± 2.1	8.3 ± 2.5
HCO3 (mmol/L)	ECLS	32.6 ± 0.9	25.9 ± 1.8*	25.5 ± 1.7*	29.2 ± 0.8	31.4 ± 2.2
	ECLS + MSC	32.8 ± 0.9	23.9 ± 1.3*	25.6 ± 1.1*	26.3 ± 2.0	27.2 ± 1.1
	Injured Control	32.3 ± 0.8	26.3 ± 1.9	26.8 ± 2.1	30.4 ± 2.1	33.0 ± 2.5
SO2 (%)	ECLS	97 ± 0	96 ± 1	94 ± 2	97 ± 1	98 ± 1
	ECLS + MSC	97 ± 0	95 ± 0	96 ± 1	93 ± 4	96 ± 2
	Injured Control	97 ± 0	93 ± 2	93 ± 1*	97 ± 1	96 ± 1
Glucose (mg/dL)	ECLS	107 ± 7	148 ± 27	93 ± 8	70 ± 8	64 ± 13*
	ECLS + MSC	93 ± 6	95 ± 10	101 ± 7	92 ± 6	83 ± 5
	Injured Control	92 ± 3	155 ± 22	97 ± 18	80 ± 10	71 ± 6
BUN (mg/dL)	ECLS	5.8 ± 0.8	8.4 ± 1.2*	20.4 ± 2.1*	20.7 ± 2.3*	25.2 ± 4.9*
	ECLS + MSC	5.2 ± 0.4	6.9 ± 0.6	15.7 ± 2.0*	20.8 ± 5.8*	21.9 ± 4.6*
	Injured Control	5.7 ± 0.8	8.4 ± 0.9*	14.9 ± 1.7*	14.0 ± 2.7*	12.9 ± 2.3
Creatinine (mg/dL)	ECLS	1.3 ± 0.0	1.6 ± 0.1*	2.2 ± 0.5	1.4 ± 0.1	3.0 ± 1.4*
	ECLS + MSC	1.3 ± 0.1	1.6 ± 0.1	1.3 ± 0.1	1.6 ± 0.3	1.4 ± 0.1
	Injured Control	1.4 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	1.3 ± 0.1

*Table 4. Arterial blood gas and blood chemistry values by group. *significant change from baseline value; † ECLS versus ECLS+MSC; § ECLS versus Injured Control; ‡ ECLS+MSC versus Injured Control, p<0.05.*

Plasma free hemoglobin (PFHb) was significantly elevated in the ECLS group compared to the ECLS+MSC and Injured Control Groups at 24 hrs, which was resolved by 48 hours. There were no other between-group differences in coagulation (Table 5). ACT and PTT were prolonged in all groups after the start of heparin administration at the post-ECLS time point.

Variable:	Group:	Time Point:					
		BL	PI	pECLS	24h	48h	72h
ACT (s)	ECLS	91 ± 2	85 ± 3	171 ± 24*	150 ± 8*	154 ± 15*	140 ± 11*
	ECLS + MSC	87 ± 2	89 ± 3	206 ± 51*	132 ± 9*	127 ± 4*	134 ± 3*
	Injured Control	85 ± 1	81 ± 2	222 ± 81*	163 ± 19*	146 ± 6*	139 ± 9*
PFHb (mg / dL)	ECLS	31 ± 2	282 ± 37*	238 ± 38*	119 ± 38* †§	37 ± 17	26 ± 4
	ECLS + MSC	45 ± 6	267 ± 30*	201 ± 28*	42 ± 10	22 ± 4	26 ± 5
	Injured Control	49 ± 9	193 ± 41*	147 ± 39*	23 ± 11	17 ± 4*	16 ± 2*
PT (sec)	ECLS	13 ± 0	14 ± 0	16 ± 1*	15 ± 1*	14 ± 1*	14 ± 1
	ECLS + MSC	14 ± 0	14 ± 0	15 ± 0*	16 ± 0*	16 ± 1*	15 ± 0*
	Injured Control	13 ± 0	14 ± 0	14 ± 0	15 ± 1*	14 ± 1*	14 ± 1
PTT (sec)	ECLS	25 ± 2	31 ± 2	145 ± 40*	70 ± 20*	59 ± 13	71 ± 27
	ECLS + MSC	32 ± 2	45 ± 4	140 ± 25*	89 ± 20*	98 ± 24*	114 ± 32*
	Injured Control	26 ± 2	38 ± 4	89 ± 42	83 ± 19*	111 ± 23*	102 ± 23*
Fib (mg / dL)	ECLS	196 ± 17	184 ± 22	162 ± 21	254 ± 36	457 ± 58*	535 ± 81*
	ECLS + MSC	174 ± 5	178 ± 11	150 ± 6*	302 ± 24*	426 ± 30*	465 ± 33*
	Injured Control	205 ± 25	203 ± 28	207 ± 26	382 ± 29*	517 ± 38*	587 ± 44*
ATIII (%)	ECLS	96 ± 2	86 ± 2	74 ± 2*	48 ± 5*	64 ± 1*	78 ± 7
	ECLS + MSC	100 ± 2	90 ± 2	80 ± 1*	55 ± 3*	58 ± 3*	67 ± 5*
	Injured Control	97 ± 2	88 ± 3	88 ± 4	54 ± 2*	59 ± 4*	71 ± 5*
TEG R (min)	ECLS	5 ± 0	3 ± 0*	4 ± 0*	6 ± 1	6 ± 1	8 ± 1*
	ECLS + MSC	5 ± 0	3 ± 0*	4 ± 0	5 ± 0	5 ± 1	6 ± 1*
	Injured Control	5 ± 0	4 ± 1	4 ± 0	6 ± 0	6 ± 0	6 ± 1
TEG Alpha (degrees)	ECLS	73 ± 1	74 ± 2*	73 ± 2	66 ± 4	70 ± 2	62 ± 5
	ECLS + MSC	76 ± 1	75 ± 1	73 ± 1	70 ± 4	73 ± 2	70 ± 3*
	Injured Control	74 ± 1	73 ± 3	71 ± 3	74 ± 1	74 ± 1	75 ± 2
TEG MA (mm)	ECLS	76 ± 1	70 ± 2*	67 ± 2*	67 ± 4*	76 ± 1	81 ± 2
	ECLS + MSC	75 ± 2	70 ± 1*	64 ± 3*	70 ± 3	74 ± 2	78 ± 2
	Injured Control	76 ± 2	70 ± 3*	68 ± 2*	77 ± 1	82 ± 2	83 ± 2*
TEG LY30 (%)	ECLS	1.0 ± 0.2	0.6 ± 0.2	0.7 ± 0.3	0.3 ± 0.1*	0.2 ± 0.1*	0 ± 0*
	ECLS + MSC	1.4 ± 0.3	0.3 ± 0.1	0.8 ± 0.3	0.4 ± 0.2*	0.3 ± 0.1*	0 ± 0*
	Injured Control	0.9 ± 0.2	0.3 ± 0.1*	0.3 ± 0.1*	0.1 ± 0.1*	0.2 ± 0.1*	0.1 ± 0.1*

*Table 5. Coagulation. *significant change from baseline value, † ECLS versus ECLS+MSC; § ECLS versus Injured Control; ‡ ECLS+MSC versus Injured Control, p<0.05.*

There were no between-group differences in complete blood count at any time point. Platelet count was significantly reduced from baseline in animals that received ECLS (ECLS and ECLS+MSC) from 24-48 hours; however, platelet count was not significantly reduced in Injured Control (Table 6).

Variable:	Group:	Time Point:				
		BL	PI	24h	48h	72h
WBC ($x10^3$/ul)	ECLS	13 ± 1	16 ± 3*	17 ± 2	17 ± 2	13 ± 2
	ECLS + MSC	13 ± 2	21 ± 3*	20 ± 4	18 ± 2	16 ± 3
	Injured Control	13 ± 1	22 ± 4*	21 ± 2*	18 ± 2	14 ± 2
RBC ($x10^3$/ul)	ECLS	5.5 ± 0.1	7.0 ± 0.3*	5.0 ± 0.3	4.2 ± 0.2*	3.7 ± 0.2*
	ECLS + MSC	5.7 ± 0.2	7.2 ± 0.2*	4.8 ± 0.1*	4.1 ± 0.1*	3.7 ± 0.2*
	Injured Control	5.5 ± 0.2	6.7 ± 0.4*	5.6 ± 0.3	4.2 ± 0.2*	4.2 ± 0.2*
HGB (g/dL)	ECLS	9.5 ± 0.3	11.8 ± 0.5*	8.7 ± 0.6	7.1 ± 0.3*	6.3 ± 0.4*
	ECLS + MSC	9.4 ± 0.3	11.4 ± 0.3*	7.9 ± 0.2*	6.8 ± 0.2*	6.1 ± 0.3*
	Injured Control	9.2 ± 0.2	10.9 ± 0.6*	8.9 ± 0.4	7.1 ± 0.4*	7.1 ± 0.3*
HCT (%)	ECLS	29 ± 1	37 ± 2*	28 ± 2	22 ± 1*	20 ± 1*
	ECLS + MSC	29 ± 1	37 ± 1*	25 ± 1*	21 ± 1*	19 ± 1*
	Injured Control	29 ± 1	35 ± 2*	28 ± 1	22 ± 1*	22 ± 1*
PLT ($x10^3$/ul)	ECLS	295 ± 22	422 ± 85*	166 ± 22*	132 ± 32*	153 ± 47*
	ECLS + MSC	298 ± 22	414 ± 37*	159 ± 21*	128 ± 24*	133 ± 30*
	Injured Control	301 ± 31	444 ± 42*	260 ± 32	271 ± 52	286 ± 50

*Table 6. Complete blood count. *significant change from baseline value p<0.05.*

Injury severity scoring of organ histology samples showed no between-group differences lung, left ventricle, liver or jejunum injury scores. Kidney injury score was significantly higher in the ECLS group versus the ECLS+MSC and Injured Control.

	Group	Mean ± Std. Error
Lung (Diffuse Alveolar Damage)	ECLS	35 ± 5
	ECLS + MSC	46 ± 3
	Injured Control	44 ± 3
Kidney	ECLS	11 ± 1 †,§
	ECLS + MSC	7 ± 1
	Injured Control	7 ± 1
Left Ventricle	ECLS	5 ± 1
	ECLS + MSC	5 ± 1
	Injured Control	5 ± 1
Liver	ECLS	12 ± 2
	ECLS + MSC	10 ± 1
	Injured Control	9 ± 1
Jejunum	ECLS	10 ± 3
	ECLS + MSC	7 ± 1
	Injured Control	7 ± 2

Table 7. Histological injury severity scores. † ECLS versus ECLS+MSC; § ECLS versus Injured Control; ‡ ECLS+MSC versus Injured Control, p<0.05.

There was no difference between groups in incidence of or time to acute kidney injury although numerical trends were observed (Table 8)

	Group	Mean ± Std. Error
AKI (%)	ECLS	17%
	ECLS + MSC	23%
	Injured Control	11%
Time to AKI (hr)	ECLS	44 ± 5
	ECLS + MSC	3 ± 0
	Injured Control	48

Table 8. Incidence of (%), and time to development (in hours) of acute kidney injury.

CONCLUSION

Use of VV-ECLS enabled reductions in mechanical ventilatory support in the ECLS and ECLS+MSC groups compared to Injured Controls; however, there was no difference in survival time or time to/degree or ARDS between the 3 groups although DAD scoring suggested the lowest injury level in the ECLS groups (not statistically different) . Further, delivery of stem cells did not demonstrate a substantial effect on mortality, development of ARDS or end-organ injury severity compared to ECLS alone and Injury Controls. This is likely due to interspecies differences but even more so, to combination of factors to include high plasma free hemoglobin in animals receiving ECLS and similar presence of acute kidney injury in all groups which was not specifically treated by means of renal replacement in these groups. Note that time to AKI was lowest and prevalence of AKI highest in ECLS+ MSC whereas kidney injury severity was highest in ECLS group which seemed to have a numerical trend for lower lung injury scores at necropsy. Other positive effects included consistent removal of CO_2 in pre vs post membrane lung samples and overall reduction in respiratory rate in animals receiving ECLS with concomitant reduction in CO_2 levels in circulating blood.
Summarizing, we observed that neither treatment improved outcomes or ARDS injury severity. This was likely due to undetermined to date effects of secondary kidney injury due to ECLS which was only partially mitigated by MSC therapy in those animals that received both. This is an important reportable observation. This work warrants further investigation of ECLS systems of different design coupled with autologous or within species use of MSC therapy delivered more locally to the lung as was investigated in the Pittsburg portion of this grant. However, in this iteration of experiments, neither therapy appeared to be useful in mitigating ARDS or multiorgan failure in this model.

3. PRODUCTS: The below listed products have been informed, in all or in part, by this work, and thus are reported as part of this effort.

Journal Articles

1. Beely BM, Campbell JE, Meyer A, Langer T, Negaard K, Chung KK, Cap AP, Cancio LC, Batchinsky AI. Electron Microscopy as a Tool for Assessment of Anticoagulation Strategies During Extracorporeal Life Support: The Proof Is on the Membrane. *ASAIO J* 2016; 62: 525-532.

2. Antebi B, Mohammadipoor A, Batchinsky AI, Cancio LC. The Promise of Mesenchymal Stem Cell Therapy for Acute Respiratory Distress Syndrome. *J Trauma Acute Care Surg* 2017; 84: 183.

3. McDaniel JS, Antebi B, Pilia M, Hurtgen BJ, Belenkiy S, Necsoiu C, Cancio LC, Rathbone CR, Batchinsky AI. Quantitative Assessment of Optimal Bone Marrow Site for the Isolation of Porcine Mesenchymal Stem Cells. *Stem Cells Int* 2017; 2017: 1836960.

4. Cannon JW, Mason PE, Batchinsky AI. Past and present role of ECMO in combat casualty care: how far will we go? *J Trauma Acute Care Surg* 2018.

5. 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*.

7. Batchinsky AI, Wyckoff R, Choi JH, Burmeister D, Jordan BS, Necsoiu C, Burkett SE, Morris MJ, Chung KK, and Cancio LC. Dynamics of acute respiratory distress syndrome development due to smoke inhalation injury: Implications for prolonged field care. *J Trauma Acute Care Surg* 87: S91-S100, 2019.

8. Choi J, Necsoiu C, Wendorff D, Jordan B, Dixon AT, Roberts T, Beely B, Cancio L, and Batchinsky A. Effects of adjunct treatments on end-organ damage and histological injury severity in acute respiratory distress syndrome and multiorgan failure caused by smoke inhalation injury and burns. *Burns epub ahead of print*: 2019.

9. Choi J, Necsoiu C, Wendorff D, Jordan B, Dixon AT, Sieck K, Roberts TR, Beely B, Cancio L, and Batchinsky A. Multiorgan Failure in ARDS: Effects of Adjunct Treatments on End-Organ Damage and Histological Injury Severity. In: *Eastern Association of Trauma*. Austin, TX: 2019.

10. Choi J, Roberts T, Wendorff D, Necsoiu C, Jordan B, Sieck K, Beely B, Cancio L, and Batchinsky A. Local Expression of HMGB1, TLR4, AQP5 and TGFB1 in ARDS Due to Smoke Inhalation and Burns in Swine Treated with Minimally Invasive Extracorporeal Life Support. In: *Military Health System Research Symposium*. Kissimmee, FL: 2019.

11. Choi J, Roberts T, Wendorff D, Necsoiu C, Jordan B, Sieck K, Beely B, Cancio L, and Batchinsky A. Systemic Expression of Damage Associate Molecule Pattern (DAMP)s After ARDS Due to Smoke Inhalation and Burns in Swine Treated with Extracorporeal Life Support. In: *Military Health System Research Symposium*. Kissimmee, FL: 2019.

12. Dado DN, Ainsworth CR, Thomas SB, Huang B, Piper LC, Sams VG, Batchinsky A, Morrow BD, Basel AP, Walter RJ, Mason PE, and Chung KK. Outcomes among Patients Treated with Renal Replacement Therapy during Extracorporeal Membrane Oxygenation: A Single-Center Retrospective Study. *Blood Purif* 1-7, 2019.

13. Hendrickson C, Linden K, Kreyer S, Beilman G, Scaravilli V, Wendorff D, Necsoiu C, Batchinsky AI, Cancio LC, Chung KK, and Lusczek ER. (1)H-NMR Metabolomics Identifies Significant Changes in Metabolism over Time in a Porcine Model of Severe Burn and Smoke Inhalation. *Metabolites* 9: 2019.

14. Miao Y, Choi JH, Chou LD, Desai V, Roberts TR, Beely BM, Wendorff DS, Espinoza M, Sieck K, Cancio LC, Brenner M, Batchinsky AI, and Chen Z. Automatic proximal airway volume segmentation using optical coherence tomography for assessment of inhalation injury. *J Trauma Acute Care Surg* 87: S132-S137, 2019.

15. Xu AL, Rodriguez LA, 2nd, Walker KP, 3rd, Mohammadipoor A, Kamucheka RM, Cancio LC, Batchinsky AI, and Antebi B. Mesenchymal Stem Cells Reconditioned in Their Own Serum Exhibit Augmented Therapeutic Properties in the Setting of Acute Respiratory Distress Syndrome. *Stem Cells Transl Med* 8: 1092-1106, 2019.

Oral Presentations

1. Antebi B, Walker III KP, Mohammadipoor A, Montgomery RK, Batchinsky AI, Cancio LC. Isolation of Bone Marrow Derived Mesenchymal Stem Cells: Effects of Aspiration Volume and Processing Technique. *Presented at Military Health System Research Symposium.* Kissimmee, FL; 2017

2. Antebi B, Walker III KP, Mohammadipoor A, Montgomery RK, Batchinsky AI, Cancio LC. Optimization of Mesenchymal-Stem-Cell Culture Conditions For Use in Clinical Trials. *Presented at Military Health System Research Symposium.* Kissimmee, FL; 2017

3. Antebi B, Walker III KP, Mohammadipoor A, Montgomery RK, Batchinsky AI, Cancio LC. Acute Lung Injury Diminishes the Secretory Potency of Bone-Marrow-Derived Mesenchymal Stem Cells. *Presented at Military Health System Research Symposium.* Kissimmee, FL; 2017

4. Batchinsky AI. Systemic Administration of Mesenchymal Stem Cells for Treatment of ARDS: nuts and bolts of a Translational Study. *Presented at 34th Annual Conference - Advances In The Care Of Critically Ill Neonates, Children, And Adults.* Snowbird, UT; 2017

5. Batchinsky AI. Extracorporeal membrane oxygenation for life-threatening bleeding in animal models. *Presented at Remote Damage Control Resuscitation Symposium.* Bergen, Norway; 2017

6. Batchinsky AI, Beely BM, Wendorff DS, Choi JH, Roberts TR, Jordan B, S., Necsoiu C, Cannon JW, Cancio LC. Minimally Invasive ECLS Reduces Minute Ventilation And Delays Development Of ARDS In A Model Of En-Route Critical Care. *Presented at 33rd Annual Children's National Medical Center Conference: ECMO and the Advanced Therapies for Respiratory Failure.* Keystone, CO; 2017

7. Beely B, Harea G, Karaliou VK, Roberts TR, Choi J, Batchinsky A. Utility of the Simplified Automated Ventilator II as a Transport Ventilator in a Combat-Relevant Model of Lung Injury. *Presented at 34th Annual Conference - Advances In The Care Of Critically Ill Neonates, Children, And Adults.* Snowbird, UT; 2017

8. Espinoza ME, Batchinsky A, Wendorff D, Beely B, Dixon AT, Lucas M, Leatherman L, Jordan B, S., Necsoiu C, Cancio L. Utility of Quantitative Analysis of Pulmonary CT Scans in Mild to Moderate ARDS. *Presented at 33rd Annual Children's National Medical Center Conference: ECMO and the Advanced Therapies for Respiratory Failure.* Keystone, CO; 2017

9. Mohammadipoor A, Roberts TR, Antebi B, Walker III KP, Rodriguez LA, Montgomery RA, Batchinsky AI, Cancio LC. Effects of Human and Porcine Mesenchymal Stem Cell-derived Conditioned Media on Coagulation and Immune Cell Function. *Presented at Military Health System Research Symposium.* Kissimmee, FL; 2017

10. Sieck KN, Beely BM, Wendorff DS, Dixon AT, Roberts TR, Jordan B, S., Necsoiu C, Lantry JH, Mason PP, Cannon JW, L.C. C, Batchinsky AI. Benchmarking CO2 Removal Efficiency During ECCO2R by Hemolung and Novalung *Presented at 33rd Annual Children's National Medical Center Symposium: ECMO and the Advanced Therapies for Respiratory Failure.* Keystone, CO; 2017

11. Wendorff DS, Choi J, Lantry JH, Roberts TR, Beely BM, Jordan B, S., Necsoiu C, Sieck KN, Espinoza ME, Cancio LC, Batchinsky AI. Utility of Optical Coherence Tomography (OCT) and Ultrasound for Diagnosis of ARDS due to Smoke

Inhalation and Burns. *Presented at 33rd Annual Children's National Medical Center Symposium: ECMO and the Advanced Therapies for Respiratory Failure.* Keystone, CO; 2017

12. Antebi B. The Effect of Acute Respiratory Distress Syndrome on Bone-Marrow Derived Stem Cells. *Presented at Fourth Annual Conference on Stem Cell Research & Regenerative Medicine.* San Antonio, TX; 2018

13. Batchinsky A. Extracorporeal Lifes Support as a Platform Intervention in Prolonged Field Care. *Presented at 35th Annual Conference - Advances in Therapeutics and Technology: Care of Critically Ill Neonates, Children, and Adults.* Snowbird, UT; 2018

14. Beely BM, Karaliou VK, Wendorff DS, Harea G, Sieck KN, Choi J, Roberts TR, Cancio LC, Sams VG, Batchinsky A. Adjunct use of Mechanical Ventilation and Veno-venous ECLS in a Model of Prolonged Field Care and Ground and High-Altitude Evacuation. *Presented at 35th Annual Conference - Advances in Therapeutics and Technology: Care of Critically Ill Neonates, Children, and Adults.* Snowbird, UT; 2018

Poster Presentations

1. Antebi B, McDaniel JS, Cancio LC, Batchinsky A. Quantitative Assessment of Optimal Bone Marrow Site for the Isolation of Mesenchymal Stem Cells. *Presented at Annual Conference on Shock*. Austin, TX; 2016.

2. Antebi B, Montgomery RK, Walker III KP, Dixon AT, McDaniel JS, Cap AP, Cancio LC, Batchinsky A. Characteristics of Mesenchymal Stem Cells from Swine with and without Acute Respiratory Distress Syndrome. *Presented at American Thoracic Society*. San Francisco, CA; 2016.

3. Antebi B, Walker III KP, Dixon AT, McDaniel JS, Rathbone CR, Necsoiu C, Lantry III JH, Cancio LC, Batchinsky A. Bedside Cell Retrieval, Characterization, and Viability for Systemic Administration of Stem Cells. *Presented at Military Health System Research Symposia*. Kissimee, FL; 2016.

4. Antebi B, Walker III KP, Mohammadipoor A, Choi J, Montgomery RK, Cancio LC, Batchinsky A. The Effect of Acute Lung Injury on the Immunomodulatory Potency of Bone-Marrow-Derived Mesenchymal Stem Cells. *Presented at Keystone Symposia*. San Francisco, CA; 2016.

5. Xu A, Walker III KP, Mohammadipoor A, Rodriguez L, Roberts TR, Batchinsky A, Cancio L, Antebi A. Mesenchymal stem cells in the setting of acute respiratory distress syndrome. *J Transl Med* 2017; 15: P10.

6. Greaney A, Choi J, Beely B, Gubbins E, Ghaedi M, Wendorff D, Roberts T, Le A, Batchinsky A, Niklason L. 1122: Regenerative Potential Of Basal Cells In Porcine Acute Respiratory Distress Syndrome Due To Trauma. *Critical Care Medicine* 2018; 46: 544.

Other Scientific Productivity.

Dr. Batchinsky was an invited contributor and briefer to MG Holcomb on war gaming of future battlefield scenarios, hosted by the United States Army Institute of Surgical Research (January 30 – February 1 2018). During this meeting Dr. Batchinsky reported on the current project and its potential role in combat casualty care, if successful.
At present Dr. Batchinsky serves as the tri-service DOD subject matter expert on extracorporeal life support where he develops programmatic research efforts for DOD
In addition, Dr. Batchinsky gave an invited lecture at the Food and Drug Administration (June 14, 2018) which was followed by a return visit by FDA to Dr. Batchinsky's USAISR and BCB laboratories in San Antonio on 17 Sept, 2018.