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TITLE: Diagnosis and Treatment of TBI and Polytrauma during Ground and High Altitude Evacuation using Liquid Biopsy and Extracorporeal Life Support

PRINCIPAL INVESTIGATOR: Andriy Batchinsky, MD

CONTRACTING ORGANIZATION: The Geneva Foundation

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
TBI alone and in combination with polytrauma and lung injury caused up to 83% of "nonsurvivable" combat-related deaths. There is no accurate diagnosis method or viable therapeutic intervention for these casualties primarily due to the severity of injury, which can be unrecognized early on. Our proposal will address these unmet needs via utilization of a model of TBI with targeted descriptors of injury severity derived from bedside cell free DNA (cfDNA) testing, and then via addition of polytrauma and lung injury with subsequent testing of therapeutic intervention via extracorporeal life support (ECLS).

15. SUBJECT TERMS
Traumatic brain injury; extracorporeal life support; cfDNA; biomarkers

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Contents

1. **INTRODUCTION:**

TBI alone and in combination with polytrauma and lung injury caused up to 83% of "nonsurvivable" combat-related deaths. There is no accurate diagnosis method or viable therapeutic intervention for these casualties primarily due to the severity of injury, which can be unrecognized early on. Our proposal will address these unmet needs via utilization of a model of TBI with targeted descriptors of injury severity derived from bedside cell free DNA (cfDNA) testing, and then via addition of polytrauma and lung injury with subsequent testing of therapeutic intervention via extracorporeal life support (ECLS). Objectives: Base Phase 1: 1) identify and characterize biomarkers specific to TBI alone and with polytrauma; compare to control animals without injury; 2) improve understanding of risk and genetic pathophysiologic factors in TBI by determining plasma levels of various injury severity markers; Phase 2: 1) develop targeted therapeutic applications for ECLS devices and clinical guidelines to improve diagnosis, stabilization, and treatment of TBI; compare therapeutic effects of ECLS with standard of care in 72 hour studies relevant to PFC; 2) develop CPGs to treat TBI with polytrauma and hemorrhage; Phase 3: assess impact of altitude, vibration, and temperature and how they affect TBI outcomes in animals with TBI and polytrauma treated with and without ECLS at high altitude.This proposal improves characterization, diagnosis and treatment of neurotrama resulting from TBI including precise characterization and individualized assessment of the specific TBI pathology; targeted diagnosis of TBI using liquid biopsy; use of treatment and stabilization/recovery protocols using brain sparing resuscitation and life preservation via extracorporeal life support and mechanical ventilation: all studied during ground and high altitude evacuation phases of en-route care. We test therapeutic interventions while carrying out digital data recording and development of knowledge products on utility of life-preservation approaches for surgical patients with massive combined trauma.

2. **KEYWORDS:**

Traumatic brain injury; extracorporeal life support; cfDNA; biomarkers

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Base Phase 1

<u>Specific Aim 1:</u> Identify cfDNA, DAMPs and other injury severity markers in TBI alone and in combination with polytrauma and bleeding (months 1-12)

<u>Major Task 1:</u> Regulatory approval (months 1-2)

- ***STATUS: started Y1Q1, 100% completed Y1Q1, however unforeseen delays required re-approval. New regulatory approval completed Y1Q3, 100% completed.***

Subtask 1: Write animal use protocol and obtain IACUC and ACURO approvals for research (months 1-2)

<u>Major Task 2:</u> Animal experiments and sample collection (months 2-12)

- ***STATUS: started Y1Q4, 7% Phase 1 animals completed***

Subtask 2: Conduct time control experiments (n=6, 48 hours duration) and collect samples for all planned assays (months 2-12)

- ***STATUS: started Y1Q4, 17% of group completed. 1 animal experiment in this group conducted 2 Feb 2020.***

Subtask 3: Conduct Group A (TBI) experiments (n=8, 48 hours duration) and collect samples for all planned assays (months 4-12)

- ***STATUS: started Y1Q4, 13% of group completed. 1 animal experiment in this group conducted 10 Feb 2020.***

Subtask 4: Conduct Group B (ARDS from trauma [bilateral pulmonary contusion]) experiments (n=8, 48 hours duration) and collect samples for all planned assays (months 4-12)

- ***STATUS: Not started, 0% of group completed***

Subtask 5: Conduct Group C (TBI + ARDS) experiments (n=8, 48 hours duration) and collect samples for all planned assays (months 4-12)

- ***STATUS: Not started, 0% of group completed***

<u>Specific Aim 2:</u> Screen transcriptional cfDNA responses modulated by TBI alone, and in combination with polytrauma and ARDS (months 2-12)

<u>Major Task 3:</u> Characterize cfDNA and severity markers (months 2-12)

- ***STATUS: started Y1Q1, 5% completed. Due to delay in animal work, banked samples from other studies (n=200) sent from Batchinsky lab to Circulogene in Y1Q4 to enable Circulogene group to continue working on cfDNA amplification and organ-specific signal identification.***

Subtask 6: Conduct cfDNA analysis by CGT method on samples collected in Specific Aim 1 (months 2-12)

Subtask 7: Conduct amplifiability examination of cfDNA samples (months 2-12)

Subtask 8: Characterize organ- and injury-specific markers and severity descriptors defined by analysis in Subtasks 6 & 7 in Groups A-C (months 2-12)

Subtask 9: Define dose-response relationship of cfDNA expression in TBI alone, ARDS from trauma alone, and TBI + ARDS (months 2-12)

Optional Phase 2 – *Not yet started*
<u>Specific Aim 3:</u> ECLS as a targeted therapeutic intervention in TBI (months 13-24)

<u>Major Task 4:</u> Therapeutic animal studies (months 13-24)

- ***STATUS: yet to start pending award mod for approval***

Subtask 10: Conduct Group D (TBI+ARDS, treatment with lung-protective ventilation and judicious fluid management) animal experiments (n=12, 72 hours in duration) (months 13-24)

Subtask 11: Conduct Group E (TBI+ARDS, treatment with VV ECLS) animal experiments (n=12, 72 hours in duration) (months 13-24)

Subtask 12: Characterize organ- and injury-specific markers and severity descriptors from Groups D & E (months 13-24)

Subtask 13: Draft and publish CPGs for ECLS usage as therapeutic intervention in TBI and ARDS from polytrauma (months 22-24)

Optional Phase 3 - *Not yet started*
Specific Aim 4: ECLS as a life-saving intervention for TBI during aeromedicalc evacuation: effects of altitude, vibration, and temperature changes as well as feasibility of VV ECLS in a model of PFC (months 25-36)

Major Task 5: Therapeutic animal studies with transport (months 25-36)
- ***STATUS: yet to start pending award mod for approval***

Subtask 14: TBI+Polytrauma group, treatment with standard of care) animal experiments (n=8, 72 hours in duration) including 7-stage ground and high altitude evacuation (months 25-36)

Subtask 15: TBI+Polytrauma group, treatment with VV ECLS) animal experiments (n=8, 72 hours in duration) including 7-stage ground and high altitude evacuation (months 25-36)

Subtask 16: Characterize organ- and injury-specific markers and severity descriptors from Groups F & G (months 25-36)

Subtask 17: Characterize effects of stressors of flight (altitude, vibration, temperature) on TBI management with VV ECLS (months 25-36)

Subtask 18: Draft and publish CPGs for VV ECLS management of TBI during aeromedical evacuation (months 34-36)

Major Task 6: Study completion (months 34-36)
- ***STATUS: yet to start pending award mod for approval***

Subtask 19: Preparation of manuscripts and technical reports on findings of study (months 34-36)
What was accomplished under these goals?
Specific Aim 1: Identify cfDNA, DAMPs and other injury severity markers in TBI alone and in combination with polytrauma and bleeding (months 1-12)

Major Task 1: Regulatory approval (months 1-2)

Subtask 1: Write animal use protocol and obtain IACUC and ACURO approvals for research (months 1-2)

Methods and results for Major Task 1 for the reporting period:

- ***Animal use protocol draft finalized and sent to local IACUC 29 March 2019. Protocol approved by local IACUC April 19, 2019 (BPTS 19-01). Protocol approved by ACURO May 16, 2019. After approval, CRO BPPTS withdrew support, necessitating new regulatory approvals and change in AAALAC accredited program. New IACUC approval received 5 November 2019 (UTSA SU-001). Protocol submitted to ACURO 12 November 2019 with expedited review requested. ACURO approval received 26 November 2019.***

<u>Major Task 2:</u> Animal experiments and sample collection (months 2-12)

- ***STATUS: started Y1Q4, 7% Phase 1 animals completed***

Subtask 2: Conduct time control experiments (n=6, 48 hours duration) and collect samples for all planned assays (months 2-12)

Subtask 3: Conduct Group A (TBI) experiments (n=8, 48 hours duration) and collect samples for all planned assays (months 4-12)

Subtask 4: Conduct Group B (ARDS from trauma [bilateral pulmonary contusion]) experiments (n=8, 48 hours duration) and collect samples for all planned assays (months 4-12)

Subtask 5: Conduct Group C (TBI + ARDS) experiments (n=8, 48 hours duration) and collect samples for all planned assays (months 4-12)

Methods and results for Major Task 2 for the reporting period:
Animal work was started on 3 February 2020. Summary of the animal experiments completed this reporting period are below:

Animal #	**Group**	**Date**	**Wt (kg)**	**Time to ARDS (hrs)**	**Survival (hrs)**
1	Time Control	3-Feb-20	57.8	24	48
2	Group A	10-Feb-20	55.8	24	48

Key Findings or Accomplishments for Specific Aim 1:
On 28 February 2020, both the UTSA IACUC and the USDA conducted surprise unannounced inspections of our new animal use space. Both of these were passed with no deficiencies noted.

<u>Specific Aim 2:</u> Screen transcriptional cfDNA responses modulated by TBI alone, and in combination with polytrauma and ARDS (months 2-12)

<u>Major Task 3:</u> Characterize cfDNA and severity markers (months 2-12)

- ***STATUS: started Y1Q1, 5% completed***

Subtask 6: Conduct cfDNA analysis by CGT method on samples collected in Specific Aim 1 (months 2-12)

Subtask 7: Conduct amplifiability examination of cfDNA samples (months 2-12)

Subtask 8: Characterize organ- and injury-specific markers and severity descriptors defined by analysis in Subtasks 6 & 7 in Groups A-C (months 2-12)

Subtask 9: Define dose-response relationship of cfDNA expression in TBI alone, ARDS from trauma alone, and TBI + ARDS (months 2-12)

Methods and results for Major Task 3 for the reporting period:

Due to the unforeseen delays in accomplishing this work, we were unable to send samples from animals in this work for analysis. This plan was established in a conference call between Dr. Batchinsky's team and Dr. Yeh and team on 3 December 2019.

To mitigate this delay, in Y1Q4 we sent banked plasma samples from other funded work to Circulogene for analysis. This was done to enable Circulogene to continue their fine-tuning of cfDNA amplification and organ-specific signal expression analysis while the Batchinsky team began animal work and sample collection.

Banked plasma samples from female Yorkshire pigs (n=32, 53.4±1.2kg) that were anesthetized and sedated (total intravenous anesthesia), and received tracheostomy, mechanical ventilation, arterial and venous catheters, fluids, and vasopressors as needed. All animals received smoke inhalation injury followed by a full-thickness flame burn over 40% total body surface area. Animals were followed under intensive care unit (ICU) conditions for up to 72 hours after injury. cfDNA and other markers were determined from samples taken at baseline (BL), at post injury (PI) and at 24, 48 and 72 hours. The concentration of plasma cfDNA was quantified directly from 20 µl of plasma using cfDNA Linear In Situ Amplification (LISA, Circulogene Theranostic, Birmingham AL, USA). Enzyme-linked immunosorbent assays (ELISA) were performed to detect systemic expression of HMGB1 (Shino-Test Corporation, Japan), TLR4 (LS-F15158, LSBio, USA), NGAL (ab207924, abcam, USA), and GFAP (LS-F22386, LSBio, USA). PfHb was measured by Plasma/Low Hb System (HemoCue©, Sweden) and TPPC was measured by Pierce BCA Protein Assay kit (23227, ThermoFisher, USA). Statistical analysis included a Spearman correlation, repeated measures analysis of variance with Tukey adjustment for multiple comparisons and receiver operating curve (ROC) analysis using SAS 9.4 (Cary, NC). Statistical significance was established at $p<0.05$

The cfDNA concentration was significantly higher at BL, PI, 24hrs and 48hrs timepoints ($p<0.05$, Figure) in non-surviving animals (n=12) vs. survivors (n=20). Circulating cfDNA levels positively correlated with NGAL, HMGB1, TPPC, GFAP and TLR4 levels for all timepoints (see table). ROC analysis demonstrated that cfDNA was significantly associated with mortality

at 72 hours with an area under the ROC of 0.73 ($p<0.0001$), with an odds ratio of 1.021 and 1.009-1.034 Confidence Interval. These results were presented at *Annual Circulating Biomarkers World Congress 2020.*

Key Findings or Accomplishments for Specific Aim 2:

Higher cfDNA concentration was seen in animals with early mortality. The level of cfDNA has a significant correlation with other DAMPs including kidney and brain damage biomarkers, indicating cfDNA concentration is an indicator of injury, not only injury severity, after SII and burns. It also assists in detecting multiorgan damage and could be useful to develop other applications in point-of-care testing to guide therapeutic interventions, aimed at reducing mortality.

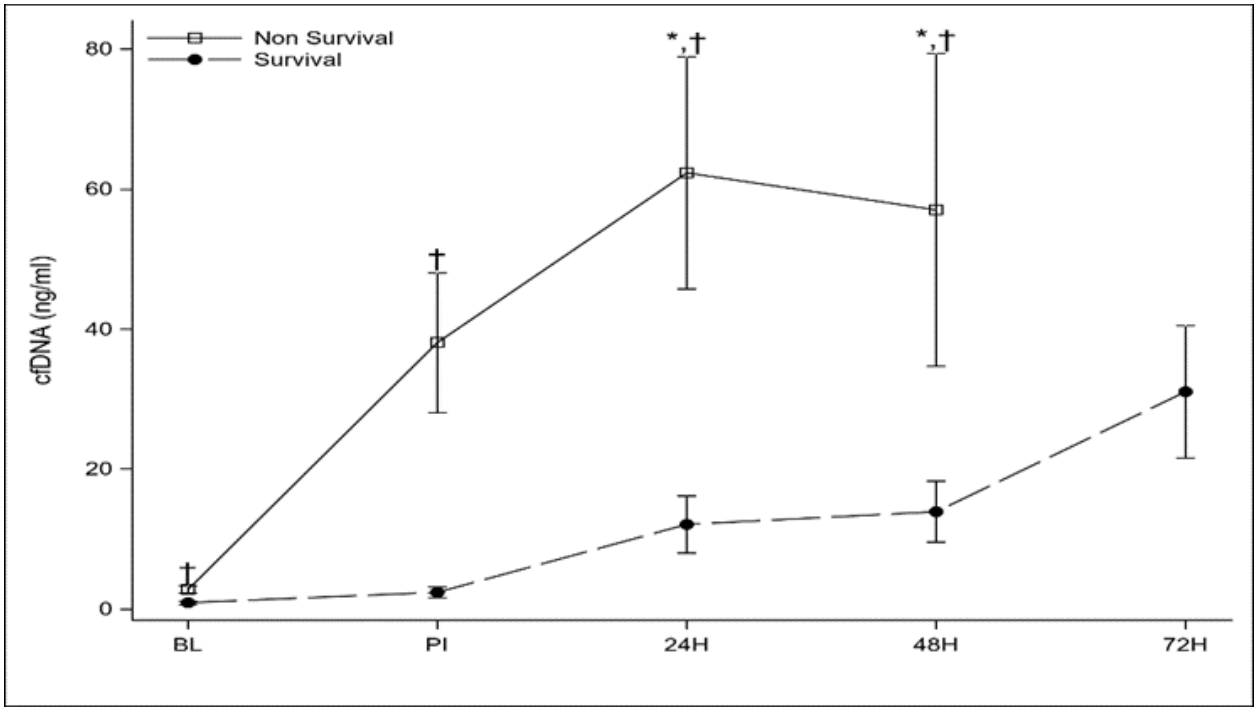

Figure 1. Cell-free DNA levels of survivor group and non-survivor group at baseline, post injury, 24 hours, 48 hours, and 72 hours. †indicates between group difference, *indicates difference vs. baseline; $p<0.05$.

Additionally, on 17 January 2020 Dr. Yeh visited the Batchinsky lab in San Antonio and provided an in-person update of their efforts.

Spearman Correlation Coefficients									
	Value	GFAP	HMGB1	NGAL	PfHb	TPPC	TLR4	cfDNA	Survival
GFAP	R		0.33285	0.06946	0.56183	0.27921	0.60610	0.32020	-0.27061
	p		0.0001	0.4305	<0.0001	0.0012	<0.0001	0.0002	0.0018
HMGB1	R	0.33285		0.37428	0.44482	-0.22352	0.44458	0.47866	-0.27871
	p	0.0001		<0.0001	<0.0001	0.0082	<0.0001	<0.0001	0.0009
NGAL	R	0.06946	0.37428		0.00713	-0.17746	0.17843	0.57356	-0.34429
	p	0.4305	<0.0001		0.9344	0.0366	0.0356	<0.0001	<0.0001
PfHb	R	0.56183	0.44482	0.00713		0.27001	0.62068	0.08564	-0.34429
	p	<0.0001	<0.0001	0.9344		0.0015	<0.0001	0.3252	<0.0001
TPPC	R	0.27921	-0.22352	-0.17746	0.27001		0.10554	-0.38987	-0.08519
	p	0.0012	0.0082	0.0366	0.0015		0.2163	<0.0001	0.3187
TLR4	R	0.60610	0.44458	0.17843	0.62068	0.10554		0.22565	-0.37039
	p	<0.0001	<0.0001	0.0356	<0.0001	0.2163		0.0080	<0.0001
cfDNA	R	0.32020	0.47866	0.57356	0.08564	-0.38987	0.22565		-0.35149
	p	0.0002	<0.0001	<0.0001	0.3252	<0.0001	0.0080		<0.0001
Survival	R	-0.27061	-0.27871	-0.35822	-0.34429	-0.08519	-0.37039	-0.35149	
	p	0.0018	0.0009	<0.0001	<0.0001	0.3187	<0.0001	<0.0001	

Figure 2. Spearman Correlation Coefficients of cfDNA with GFAP, HMGB1, NGAL, TPPC and pfHb on all time points Correlation indicated by shading.

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

- Dr. Jae Choi attended the 2020 Circulating Biomarkers World Congress, Coronado Island, California February 17-18 2020.

How were the results disseminated to communities of interest?

Dr. Choi presented a poster on the above results:

Choi J, Yeh CH, Roberts T, Wendorff D, Beely B, Harea G, Batchinsky A. Early Post Injury Systemic Cell-free DNA is Associated with Survival in 72-Hour Intensive Care Unit Study in Swine with Smoke Inhalation and Burns. Circulating Biomarkers World Congress. Coronado Island, California; 2020.

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

We have scheduled animal experiments throughout the next reporting year to continue this work.

To be ready and achieve the Year 2 goals, the Circulogene team is setting out a new assay development and validation plan to identify the sources of elevated cfDNA, i.e., injured organs. We plan to tackle this issue from two angles: by cfDNA epigenetic sequence pattern; and by cfRNA expression mapping. These two approaches can only be made possible through Circulogene's validated Linear In Situ Amplification (LISA) technology, thanks to its high yield of cfDNA/cfRNA.

Besides cfDNA/cfRNA, the most important materials in this study are reference DNA and RNA from each organ that we're targeting - Brain, Lung, Liver, kidney, Spleen, Small Intestine and Blood. These organ-specific DNAs/RNAs will serve as "genetic maps" to tell us where the elevated cfDNA/cfRNA are coming from.

Swine organ-specific DNAs/RNAs and assay reagents/buffers will be procured in the next reporting period. We expect to start assay development in early May 2020, and hopefully we will have preliminary data available in June/July.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?
Nothing to report this period.

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.

5. **CHANGES/PROBLEMS:**

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
Soon after obtaining ACURO approval for the study to perform animal research the contracted provider for animal services, Bridge PTS, refused to honor the IACUC and ACURO approved protocol to support experiments on this study and withdrew support.

This led to a halt in study execution during which we identified a new vendor for animal services the University of Texas San Antonio AAALAC accredited program to support this and any other upcoming study/grant/animal work from our laboratory.

Specifically, animal delivery, veterinary oversight, health check and all other mandated activities are now conducted by UTSA staff. A fully updated redone budget and SOW were sent to the Science and contracting officer and were approved for execution. No changes to the overall amount of funding was needed.

Our laboratory also passed successfully an unannounced visit from a local USDA official whom inspected the procedure room and intensive care unit room and found them acceptable for all of the proposed work. This was a prerequisite to submission and considering the animal use protocol by the UTSA IACUC.

On 12 July 2019 the PI presented the protocol to the IACUC at UTSA and answered questions from the members. We were notified 22 July 2019 that the protocol is expected to be approved pending minor adjustments and changes which we since completed. The only current delay is

awaiting official letter from the IACUC and appointment letter for PI as adjunct UTSA investigator (without pay).

In an email dated 26 July 2019 the way forward was detailed to the science officer and contract specialist and both approved the course of action going forward.

According to the new approved research plan the animal studies in our lab and program from now on will be conducted at an intensive care unit (ICU) in an adjacent building where KBR is located and has signed an agreement with our lab to support these studies. The budget was redone to accommodate this change and additional equipment which was required according to the AAALAC accreditation of equipping the room with a self-contained HVAC unit and air filter. The latter is the new equipment item called the BioBubble.

The BioBubble was installed on 7 October 2019, and the self-contained HVAC air handler units were certified 4 December 2019. Equipment was moved into the BioBubble pending completion of all necessary regulatory pieces to resume animal work.

An application was filed with the DEA 18 October 2019 for license to purchase of required controlled substances, and the license was granted to Dr. Batchinsky on 11 December 2019. This function was filled by the previous CRO; since dissolution of that relationship Dr. Batchinsky will now fulfill this role.

We began animal work under this new arrangement on 3 February 2020.

We requested an extension to complete the planned Y1 work on this project, and received said extension effective 6 February 2020. At present we are scheduled to complete all animals by the deadline of the extension and do not envision any delays.

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
None required to complete SOW.

Significant changes in use or care of vertebrate animals.
None.

Significant changes in use of biohazards and/or select agents
None required to complete SOW.

6. **PRODUCTS:**

Publications, conference papers, and presentations

Journal publications.

Choi J, Necsoiu C, Wendorff D, Jordan B, Dixon AT, Roberts T, Beely B, Cancio L, **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* 2019; 45: 1765-1774.

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

None this reporting period.

Other publications, conference papers, and presentations.

Oral presentations:

1. Choi J, Necsoiu C, Wendorff D, Jordan B, Dixon A, Roberts T, Beely B, Cancio L, **Batchinsky A.** Effects of Systemic Mesenchymal Stem Cell Therapy on End Organ Injury Severity in ARDS. RegenMedSA. San Antonio, TX; 2020.

Posters:

1. Choi J, Roberts T, Wendorff D, Necsoiu C, Jordan B, Sieck K, Beely B, Cancio L, **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. Military Health System Research Symposium. Kissimmee, FL; 2019.

2. Choi J, Roberts T, Wendorff D, Necsoiu C, Jordan B, Sieck K, Beely B, Cancio L, **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. Military Health System Research Symposium. Kissimmee, FL; 2019.

3. Garcia I, Willis RP, Lee J, Roberts T, Wendorff D, Beely B, Harea G, Sieck K, **Batchinsky A**, Choi J. Injury Severity Validation with Bronchoalveolar Lavage Cell Analysis: Expression of HMGB1 and TLR4 After Smoke Inhalation Injury and Burns in Swine Treated with Extracorporeal Life Support. Military Health System Research Symposium. Kissimmee, FL; 2019.

4. Choi J, Yeh CH, Roberts T, Wendorff D, Beely B, Harea G, **Batchinsky A.** Early Post Injury Systemic Cell-free DNA is Associated with Survival in 72-Hour Intensive Care Unit Study in Swine with Smoke Inahaltion and Burns. Circulating Biomarkers World Congress. Coronado Island, California; 2020.

Website(s) or other Internet site(s)

None this reporting period.

Technologies or techniques

None this reporting period.

Inventions, patent applications, and/or licenses

None this reporting period.

Other Products

None this reporting period.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Andriy I. Batchinsky, MD
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-8601-2827
Nearest person month worked:	1.8
Contribution to Project:	Overseeing and carrying out the project protocol, collecting and analyzing data, and preparing and finalizing manuscripts and reports.

Name:	Jae Choi, PhD
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	2.4
Contribution to Project:	Carrying out the project protocol, carrying out histological assessment and ELISA assays, and preparing manuscripts and reports.

Name:	Teryn Roberts
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	2
Contribution to Project:	Carrying out histology and pathology protocols, overseeing biochemical assays, assisting in data collection and management, and coordinating the preparation of manuscripts and reports.

Name:	Brendan Beely
Project Role:	Research Coordinator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.8
Contribution to Project:	Performing routine laboratory procedures, assisting with tissue histology procedures, implementing study protocols, and preparing manuscripts and reports.

Name:	Dan Wendorff
Project Role:	Laboratory Manager

Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	2.3
Contribution to Project:	Performing routine laboratory procedures, assisting with tissue histology procedures, implementing study protocols, preparing manuscripts and reports, and overseeing Lab. Tech.

Name:	George Harea
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Assisting with histology and pathology protocols, assisting with biochemical assays, assisting in data collection and management, assisting in the preparation of manuscripts and reports.

Name:	Isabella Garcia
Project Role:	Laboratory Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.5
Contribution to Project:	Assisting with large animal handling (anesthesia/sedation, subject monitoring, and drug administration), assisting with data collection and interpretation.

Name:	Hailee Alaniz
Project Role:	Laboratory Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.4
Contribution to Project:	Assisting with large animal handling (anesthesia/sedation, subject monitoring, and drug administration), assisting with data collection and interpretation.

Name:	Clayton Smith
Project Role:	Laboratory Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.3
Contribution to Project:	Assisting with large animal handling (anesthesia/sedation, subject monitoring, and drug administration), assisting with data collection and interpretation.

Name:	Catherine Kaputska
Project Role:	Laboratory Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.4
Contribution to Project:	Assisting with large animal handling (anesthesia/sedation, subject monitoring, and drug administration), assisting with data collection and interpretation.

Name:	Robert Willis
Project Role:	Laboratory Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.2
Contribution to Project:	Assisting with large animal handling (anesthesia/sedation, subject monitoring, and drug administration), assisting with data collection and interpretation.

Name:	Ji Lee
Project Role:	Laboratory Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.1
Contribution to Project:	Assisting with large animal handling (anesthesia/sedation, subject monitoring, and drug administration), assisting with data collection and interpretation.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

None this reporting period.

What other organizations were involved as partners?

Organization Name: Circulogene Theranostics
Location of Organization: Birmingham, Alabama
Partner's contribution to the project

- Collaboration
- Sample Analysis

8. **SPECIAL REPORTING REQUIREMENTS**

Quad Chart: see attached.