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TITLE: Extracorporeal Nerve Agent Detoxification: A Novel Approach Combining 2 High-Priority DOD Medical Programs for Battlefield Medicine

PRINCIPAL INVESTIGATOR: Andriy Batchinsky, MD

CONTRACTING ORGANIZATION: The Geneva Foundation, Tacoma, WA

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Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)

Andriy Batchinsky

5d. PROJECT NUMBER

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5f. WORK UNIT NUMBER

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

GENEVA FOUNDATION, THE
917 PACIFIC AVE STE 600
TACOMA WA 98402-4437

8. PERFORMING ORGANIZATION REPORT NUMBER

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13. SUPPLEMENTARY NOTES

14. ABSTRACT Recent world events have shown that our near-peer adversaries are willing and able to deploy CWNAs. Consideration of how to fight in a complex kinetic environment intermixed with chemical threats is paramount to winning in future MDO against key belligerents. CWNAs are extremely toxic compounds that bind to and inhibit acetylcholinesterase[13]. This inhibition causes an excess of the neurotransmitter acetylcholine at the synapse, resulting in a cholinergic crisis. Symptoms of CWNA exposure include hypersecretions, miosis, loss of consciousness, flaccid paralysis, and seizures. CWNAs complicate battlefield trauma and no current technologies in the US medical treatment arsenal are designed to treat combined injury (trauma and CWNAs). Current treatments for CWNA exposure by itself include administration of atropine to control peripheral secretions, pralidoxime chloride to reactivate acetylcholinesterase, and diazepam or midazolam to control the occurrence of seizures. Although timely administration of these countermeasures can increase survival, they do not ameliorate all seizure activity and cannot prevent nerve agent-induced neuronal and cardiac damage. In addition, field treatment for CWNA exposure requires re-administration of these three therapeutics based on clinical signs and symptoms. Current development for prolonged protection against CWNA exposure utilizes a protein-based scavenger in circulation to either bind (stoichiometric) or hydrolyze (catalytic) CWNAs. But, the circulatory stability of bioscavengers, immunogenicity of catalytic bioscavengers, and large bioavailable concentrations needed for stoichiometric bioscavengers would limit their utility during direct field intravenous application.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRDC
a. REPORT U	**b. ABSTRACT** U	**c. THIS PAGE** U	UU	12	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

1. INTRODUCTION:

This project originated from the 2018 War Gaming Exercise conducted at the USA ISR. One of the important outcomes of that meeting was the realization that future conflicts with near-peer adversaries will involve medical care at point of injury. Since the MRDC leadership identified extracorporeal life support (ECLS) as one of the main interventions in future conflicts, the decision was made to consider a collaborative study between the Autonomous Reanimation and Evacuation (AREVA) Research Program led by Dr. Batchinsky and the ICD, PI Tamara Otto PhD with the goal of developing a novel materiel solution for continuous scavenging and inactivation of toxic chemical agents using a modified and special designed ECLS/dialysis unit. The project has been funded for a period of one year and the below summary pertains to accomplishments in that timeframe which were to a significant extent affected by contractual delays and COVID-19 slowdown in research activities. The main objective for the funded period was that the two teams will work with extensive bilateral visits and interactions to develop the ex vivo circulation system prototype in both labs but primarily in the Otto lab, which would enable combination therapy via veno-venous ECLS and integrated dialysis to incorporate CWNA mitigation capabilities. This work is in progress and will be finished with existing funds.

2. KEYWORDS:

Extracorporeal circulation, toxin removal.

3. ACCOMPLISHMENTS:

Please see the modified SOW below, adjusted from the original SOW to reflect the adjusted funding levels.

What were the major goals of the project?

Specific Aim 1: Determine if CWNA passed over a renal dialysis cartridge will transfer into the dialysate.	**Timeline**	**ICD**	**AREVA**
Major Task 1: Develop an *ex vivo* circulation testing platform	**Months**		
Subtask 1 – Purchase equipment for testing platform.	**1**	**X**	**X**
Subtask 2 – Assemble testing platform.	**2**	**X**	**X**
Subtask 3 – Perform 6-hour circulation study. • Swine blood will circulate through the testing platform, and integrity of blood will be verified by blood gas analysis, complete blood count and basic coagulation panel.	**2-3**	**X**	**X**
Milestone(s) Achieved: Validate *ex vivo* circulation testing platform			**X**
Major Task 2: Administer CWNA to the *ex vivo* circulation testing platform			
Subtask 1 – Add CWNA to blood compartment of testing platform. • CWNA will be added to system. Aliquots will be removed periodically and examined for the presence of CWNA in the dialysate.	**3-6**	**X**	
Milestone(s) Achieved: Demonstrate use of *ex vivo* circulation testing platform to assess transfer of CWNA to dialysate			
Specific Aim 2: Evaluate feasibility of extracorporeal bioscavenging system in six-hour *ex vivo* blood circulation model.			
Major Task 3: Administer bioscavenger to *ex vivo* testing platform			

Subtask 1 –Stoichiometric bioscavenger will be obtained from a commercial source • BuChE does not functionally express in *E. coli*; *Trichoplusia ni* expression platform will be utilized via a commercial source.	**1-6**	**X**	
Subtask 2 – Catalytic bioscavenger will be expressed and purified from *E. coli* in quantities for *in vitro* testing • Bacteria will be transformed with plasmid encoding expression of catalytic bioscavenger • Bacteria will be expanded for expression of catalytic bioscavenger • Catalytic bioscavenger will be purified using IMAC.	**1-6**	**X**	
Subtask 3 – Bioscavenger will be added to the *ex vivo* testing platform • CWNA and bioscavenger will be added to the system. Aliquots will be removed periodically and examined for the presence of CWNA in the dialysate.	**7-12**	**X**	
Milestone(s) Achieved: Demonstrate use of *ex vivo* circulation testing platform to assess capacity of bioscavenger to reduce CWNA levels in dialysate			

What was accomplished under these goals?

In this reporting period we carried out experiments using donor blood and 2 configurations of the extracorporeal circuitry which was further optimized from last years' report as outlined in Figure 1 below. Specifically, we modelled a "real life scenario" of a combat casualty needing Multifactorial life support (hence addition of a cardiovascular and circulatory support membrane (XLung) coupled with a dialysis circuit (NxStage) and the scavenger circuitry. This comprehensive set up was tested in t and the technical data is provided below in this report.

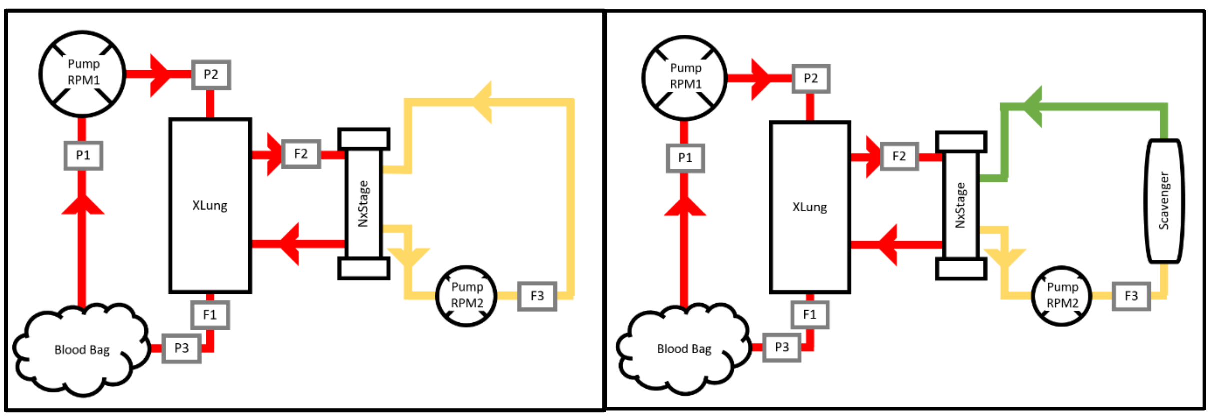

Figure 1. Schematics of of Control (left panel) and Scavenger (right panel) groups. Main XLung circuit has pressure sensors P1, P2 and P3 for measurements at different points in the circuit. Flow meter F1 measures the blood flow of the circuit. RPM 1 indicates pump speed for flow through main circuit pump. The NxStage circuit branches off the Xlung and the blood flow through the circuit is measured by flow meter F2. The scavenger circuit is branched off the NxStage and it is controlled by an independent pump. RPM 2 indicates pump speed for flow through second pump of Control and Scavenger circuits. F3 indicates blood flow through the scavenger circuit. In the Scavenger group, a scavenger device is placed in line after the pump and F3.

		Timepoint:				
Variable	**Group**	**BL**	**BL2**	**1h**	**2h**	**3h**
XLung Flow (F1)	Control	3.45 ± 0.28	3.60 ± 0.37	3.60 ± 0.37	3.60 ± 0.37	3.60 ± 0.38
	Scavenger	3.60 ± 0.09	3.83 ± 0.28	3.75 ± 0.34	3.70 ± 0.33	3.73 ± 0.29
RPM 1	Control	5275.00 ± 429.21	5525.00 ± 486.54	5525.00 ± 486.54	5525.00 ± 486.54	5575.00 ± 495.45
	Scavenger	5575.00 ± 170.93	4450.00 ± 1083.11	5900.00 ± 448.61	5875.00 ± 451.91	6075.00 ± 355.98
P1 (mmHg)	Control	-38.00 ± 15.05	-44.00 ± 15.04	-43.75 ± 15.25	-43.50 ± 15.21	-45.25 ± 15.32
	Scavenger	-46.50 ± 4.01	-59.25 ± 11.39	-55.50 ± 12.82	-54.50 ± 13.29	-57.50 ± 11.95
P2 (mmHg)	Control	115.00 ± 8.70	126.00 ± 15.00	126.25 ± 14.84	126.50 ± 15.17	128.75 ± 15.20
	Scavenger	121.25 ± 6.44	149.25 ± 16.54	140.50 ± 21.33	140.50 ± 21.78	148.75 ± 17.37
P3 (mmHg)	Control	50.50 ± 11.86	71.50 ± 6.41	71.25 ± 6.20	71.00 ± 6.31	72.25 ± 5.75
	Scavenger	63.50 ± 3.49	82.75 ± 9.63	79.25 ± 11.99	77.75 ± 12.06	80.00 ± 9.57
Blood Temp (deg C)	Control	37.03 ± 0.27	36.63 ± 0.52	36.60 ± 0.45	36.55 ± 0.45	36.60 ± 0.46
	Scavenger	36.53 ± 0.22	36.43 ± 0.27	36.35 ± 0.16	36.55 ± 0.18	36.83 ± 0.13
Sweep Gas Flow (L/min)	Control	13.00 ± 0.00	13.00 ± 0.00	13.00 ± 0.00	13.00 ± 0.00	13.00 ± 0.00
	Scavenger	13.00 ± 0.00	13.00 ± 0.00	13.00 ± 0.00	13.00 ± 0.00	13.00 ± 0.00
Air Flow Rate (L/min)	Control	12.64 ± 0.03	12.64 ± 0.03	12.64 ± 0.03	12.64 ± 0.03	12.64 ± 0.03
	Scavenger	12.68 ± 0.04	12.68 ± 0.04	12.68 ± 0.04	12.68 ± 0.04	12.68 ± 0.04
CO_2 Flow Rate (L/min)	Control	0.36 ± 0.03	0.36 ± 0.03	0.36 ± 0.03	0.36 ± 0.03	0.36 ± 0.03
	Scavenger	0.33 ± 0.04	0.33 ± 0.04	0.33 ± 0.04	0.33 ± 0.04	0.33 ± 0.04
ACT (s)	Control	999.00 ± 0.00	799.00 ± 0.00	999.00 ± 0.00	--	838.50 ± 113.49
	Scavenger	999.00 ± 0.00	999.00 ± 0.00	--	--	999.00 ± 0.00
pH	Control	7.38 ± 0.03	7.42 ± 0.02	7.41 ± 0.02	7.43 ± 0.02	7.44 ± 0.02
	Scavenger	7.40 ± 0.04	7.38 ± 0.03	7.17 ± 0.08	7.27 ± 0.06	7.26 ± 0.08
pCO_2 (mmHg)	Control	35.00 ± 1.37	35.50 ± 2.02	35.50 ± 1.89	35.75 ± 1.67	36.25 ± 2.25
	Scavenger	35.50 ± 1.35	35.75 ± 1.92	36.00 ± 1.06	36.25 ± 1.78	39.00 ± 1.89
pO_2 (mmHg)	Control	139.25 ± 0.96	136.00 ± 1.12	134.25 ± 1.56	137.50 ± 1.95	135.25 ± 0.89
	Scavenger	139.00 ± 1.80	139.00 ± 1.12	135.25 ± 0.41	134.50 ± 2.19	135.00 ± 1.70
K^+ (mmol/L)	Control	8.13 ± 0.41	8.28 ± 0.40	8.58 ± 0.39	9.03 ± 0.51	9.30 ± 0.54
	Scavenger	10.80 ± 1.53	10.00 ± 1.64	8.13 ± 1.33	8.13 ± 1.74	9.68 ± 2.86
Glucose (mmol/L)	Control	104.00 ± 2.29	117.75 ± 10.47	115.00 ± 9.10	109.75 ± 7.99	107.25 ± 7.08
	Scavenger	88.00 ± 20.07	90.00 ± 22.38	87.00 ± 22.07	81.50 ± 17.54	76.50 ± 18.67
Lactate (mmol/L)	Control	8.60 ± 0.97	9.13 ± 0.97	9.48 ± 1.15	10.25 ± 1.13	10.98 ± 1.23

	Scavenger	10.65 ± 0.91	9.65 ± 0.81	9.30 ± 0.77	9.75 ± 0.96	10.10 ± 0.85
tHb (g/dL)	Control	10.03 ± 1.18	10.50 ± 1.05	10.53 ± 1.05	10.55 ± 1.05	10.70 ± 1.00
	Scavenger	10.53 ± 1.05	11.18 ± 0.69	10.75 ± 0.64	10.58 ± 0.56	10.78 ± 0.72
BE (mmol/L)	Control	-4.35 ± 1.41	-1.80 ± 1.31	-2.40 ± 1.07	-0.63 ± 0.91	-0.08 ± 0.51
	Scavenger	-2.45 ± 2.38	-4.23 ± 1.26	-14.20 ± 4.13	-9.07 ± 3.34	-8.40 ± 6.15
HCT (%)	Control	30.00 ± 3.52	31.75 ± 3.15	31.75 ± 3.11	31.75 ± 3.15	32.00 ± 2.94
	Scavenger	31.50 ± 3.05	33.50 ± 2.19	32.25 ± 1.88	31.75 ± 1.75	32.50 ± 2.08
WBC (x10^3 / uL)	Control	8.15 ± 1.77	8.31 ± 1.72	--	--	5.46 ± 1.47
	Scavenger	10.54 ± 1.22	10.30 ± 1.29	--	--	8.12 ± 0.79
PLT (x10^3/uL)	Control	133.50 ± 19.59	127.50 ± 16.00	--	--	142.75 ± 12.07
	Scavenger	130.75 ± 21.63	147.75 ± 7.83	--	--	150.00 ± 3.56
PfHb (mg/dL)	Control	127.50 ± 29.66	125.00 ± 28.83	--	--	162.50 ± 22.74
	Scavenger	213.33 ± 35.38	175.00 ± 17.68	--	--	246.67 ± 5.44
NxStage Flow (F2) (mL/min)	Control	392.00 ± 11.20	374.75 ± 32.77	371.50 ± 34.69	362.00 ± 34.83	365.50 ± 33.16
	Scavenger	390.25 ± 13.07	403.25 ± 28.34	396.50 ± 31.95	379.00 ± 34.01	373.75 ± 25.95
Scavenger Flow (F3) (mL/min)	Control	--	49.50 ± 0.75	49.00 ± 0.61	48.50 ± 0.75	48.25 ± 0.96
	Scavenger	--	49.25 ± 0.41	49.00 ± 0.71	48.00 ± 2.03	41.75 ± 4.44
RPM 2	Control	--	37.00 ± 0.47	36.33 ± 0.27	36.67 ± 0.27	36.67 ± 0.27
	Scavenger	--	36.50 ± 0.35	37.50 ± 1.06	37.50 ± 1.06	37.50 ± 1.06

Table 1 Data reported as mean±standard error. Control circuits (n=4) had no test (Scavenger) cartridge, but a matched tubing loop; Scavenger circuits (n=4) included same configuration as Control circuits + Scavenger cartridges. BL indicates initial baseline measurements whereas BL2 indicates after beginning flow through the Control and Scavenger circuits. P1, P2, and P3 are circuit pressures. ACT: Activated Clotting Time; tHb: Total Hemoglobin; BE: Base Excess; HCT: Hematocrit; WBC: White Blood Cell; PLT: Platelet; PfHb: Plasma Free Hemoglobin. RPM2 indicates pump speed for flow through Control and Scavenger circuits. – indicates data not available (either samples not drawn at that time point or flow through Scavenger cartridges not yet started).

Table 1 lists the raw data from the experiments and provides a complete data set for performance of the system ex-vivo. Since our partners at ICD are no longer working on this project, we at AREVA, nonetheless, continue to work towards a viable prototype. A full assessment of the raw data and further interpretation will be provided in the final report of this project.

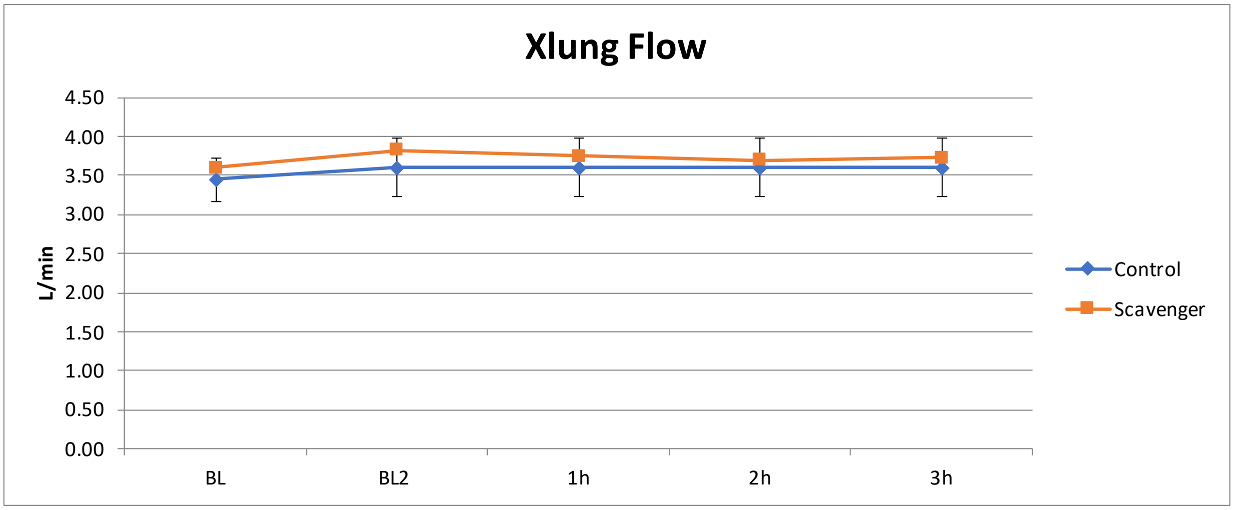

Figure 2 Blood flow through main extracorporeal circuit. Data reported as mean±standard error. Control circuits (n=4) had no test cartridge, but a matched tubing loop; Scavenger circuits (n=4). BL indicates initial baseline measurements whereas BL2 indicates after beginning flow through the test cartridges.

Figure 2 contains mean blood flow through the Xlung and Control and Scavenger circuits demonstrating identical flow patterns and the viability of the overall concept.

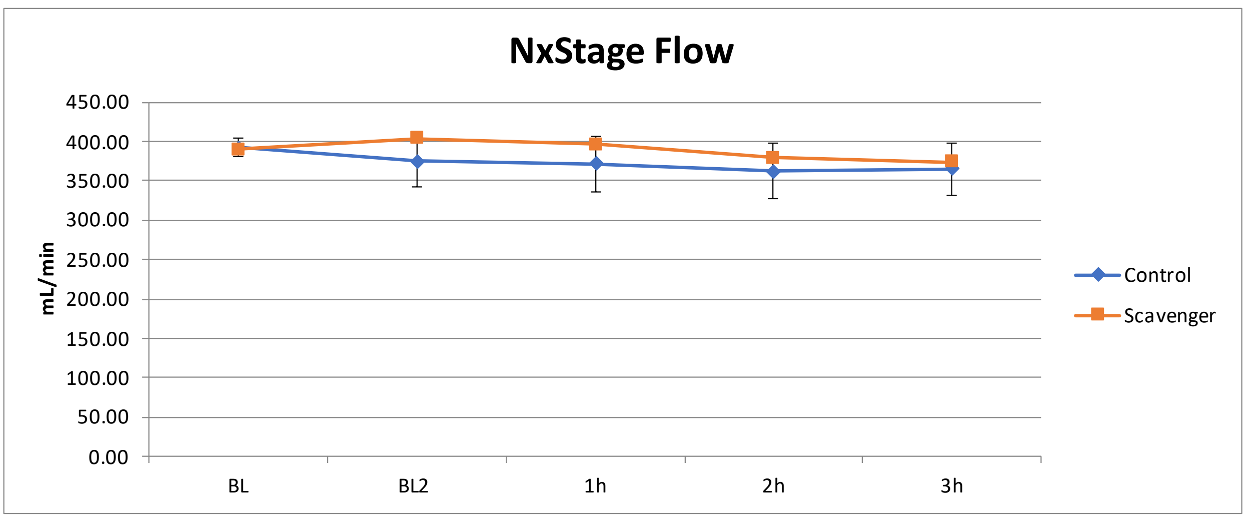

Figure 3. Blood flow through NxStage renal support membrane. Data reported as mean±standard error. Control circuits (n=4) had no test cartridge, but a matched tubing loop; Treat circuits (n=4) included test cartridges. BL indicates initial baseline measurements whereas BL2 indicates after beginning flow through the test cartridges.

Figure 3 contains mean blood flow through the Control and Scavenger circuits alone demonstrating identical flow patterns and the viability of the overall concept and circuitry operation with the Xlung flow in place and set at 3.5 Liters per minute with resultant blood flows to the Control and Scavenger circuits in the 350-400 ml/min blood flow range.

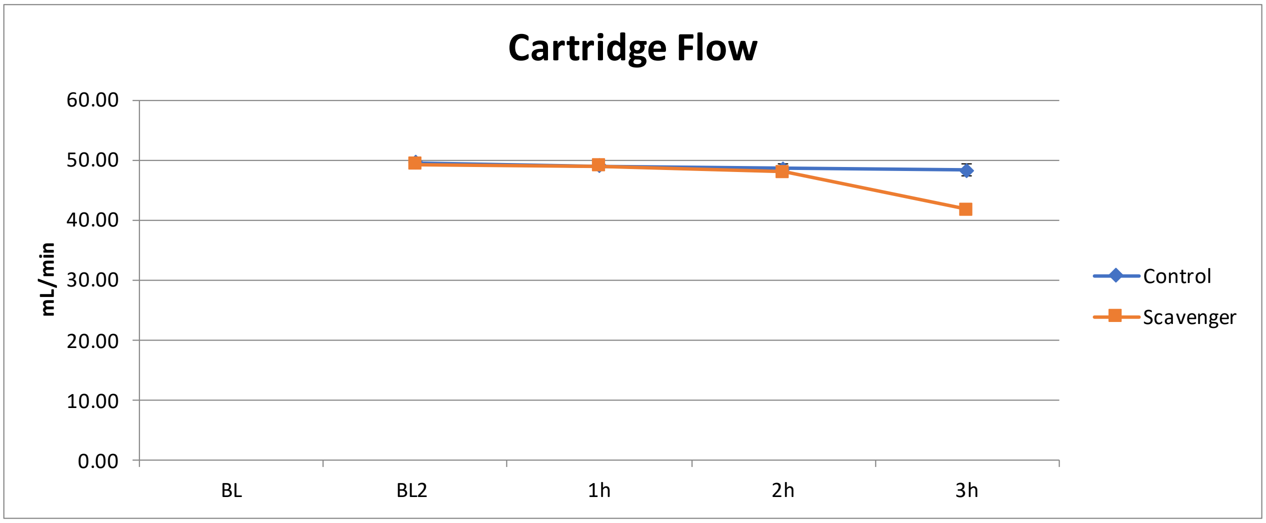

Figure 4. Blood flow through test cartridge circuit. Data reported as mean±standard error. Control circuits (n=4) had no test cartridge, but a matched tubing loop; Treat circuits (n=4) included test cartridges. BL indicates initial baseline measurements whereas BL2 indicates after beginning flow through the test cartridges.

Figure 4 contains mean blood flow through the Control and Scavenger circuits alone demonstrating identical flow patterns and the viability of the overall concept and circuitry operation with the Xlung flow in place and set at 3.5 Liters per minute with resultant blood flows to the Control and Scavenger circuits in the 350-400 ml/min blood flow range. At these settings the optimal blood flow through the Scavenger circuit alone is in the 40-50 ml/min range, confirming viability of the overall operation of the system ex-vivo.

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

We trained (3) medical students from the University of the Incarnate Word School of Osteopathic Medicine, San Antonio, Texas, in laboratory procedures and ex-vivo experiment conductance.

The methods developed here are also useful for our PhD candidate George Harea as preparation for his thesis.

How were the results disseminated to communities of interest?

Dr. Batchinsky reported part of the methods in his presentation at MHSRS 2022.

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

We will formally analyze the data and provide a final report on the performance of the ex-vivo system and refer to its potential use in in-vivo conditions as a universal system to remove toxic substances from circulating blood.

4. IMPACT:

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

This system under development, is a prototype of a pathogen or toxin agnostic removal system to be used in in -vivo studies in future separately funded efforts. It constitutes a previously not described concept of concomitant lung and cardiovascular support and parallel removal of toxic substances from blood via infusion of an antidote or addition of a specific scavenger system.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report in this period. Previously reported challenges have been resolved.

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

Nothing to report.

Changes that had a significant impact on expenditures

Other than the ICD being defunded from continuation of this collaboration, nothing else to report.

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

None

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS:

Publications, conference papers, and presentations

Nothing to report.

Journal publications.

Nothing to report.

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

Nothing to report.

Other publications, conference papers and presentations.
Nothing to report.

Website(s) or other Internet site(s)
Nothing to report.

Technologies or techniques
Nothing to report.

Inventions, patent applications, and/or licenses
Nothing to report.

Other Products
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Andriy Batchinsky, MD
No Change

Name: Teryn Roberts, PhD
Project Role: Co-PI
Researcher Identifier (e.g. ORCID ID): ORCID 0000-0002-2460-6432
Nearest person month worked:0.5
Contribution to Project: Overseeing conduct of the study, supervising study execution, data and sample collection and analysis, experiment planning, report and manuscript preparations.

Name: George Harea
Project Role: Research Associate II
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 3.8
Contribution to Project: Assisting with ex vivo circulation study execution and post-circulation material analysis, biosample processing

Name: Gregory V Williams
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.6
Contribution to Project: Assisting with data collection and interpretation.

Name: Bridget Lee
Project Role: Administrative Assistant

Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.3
Contribution to Project: Assisting with the coordination of meetings with collaborator, meeting minutes, shipping samples.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Dr. Jae Choi, PhD if no longer an employee of the Geneva Foundation and submitted his resignation on September 30, 2021 with a last day of October 22, 2021. Dr. Choi is no longer serving as a Co-I for this project.

What other organizations were involved as partners?
Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.
N/A