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TITLE: Extracorporeal Nerve Agent Detoxification: A Novel Approach Combining 2

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CONTRACTING ORGANIZATION: The Geneva Foundation

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

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13. SUPPLEMENTARY NOTES

14. ABSTRACT: Future multi-domain operations (MDO) against near-peer adversaries require new technologies and approaches to prolonged medical care of battlefield casualties. The development of field-deployable extracorporeal life support (ECLS) has been proposed to support single and multiorgan failure in combat applications, as well as ensure survivability at point of need and during en-route care. Recent events have shown that our near-peer adversaries are willing and able to deploy chemical warfare nerve agents (CWNAs). Current research to support prolonged protection against CWNAs utilizes bioscavengers, enzymes with the capacity to bind to or hydrolyze CWNAs, thereby rendering them harmless. While bioscavengers have proven effective at protecting against CWNA exposure in a laboratory setting, the regulatory and production pathway for non-immunogenic bioscavengers has proven difficult. The development of a field-deployable ECLS provides a novel platform for battlefield organ failure regardless of whether the injury is from trauma or CWNA exposure. In this context, ECLS can support respiration and cardiovascular function and, at the same time, serve as a platform for mitigation and treatment of CWNA intoxication. Removal of CWNAs from the blood through dialysis via ECLS and dialysate bioscavenging will reduce the CWNA exposure in addition to maintaining a steady level of protection during prolonged field care in a chemical environment. Since the bioscavenger will not enter the patient's circulatory system, the circulatory stability, immunogenicity, and bioavailability limitations of the current bioscavenger approach are reduced or eliminated. As acute exposure to CWNAs can cause respiratory failure, ECLS is likely to be used to treat casualties that were also exposed to CWNAs, and adding a CWNA treatment to the system increases the probability of warfighter survival. Taken together, this proposal directly addresses the Combat Casualty Care Research Program Focus Area.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	9	**19b. TELEPHONE NUMBER** *(include area code)*

Preface. 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. A status update as of 31 Aug 2022, is provided below. Importantly, the remainder of the funds will be used to execute the ex vivo experiments with donor blood as originally proposed.

1. **Accomplishments: Please see the original SOW below. The 'X" in track changes denote work to be performed with the remainder of the funds as we continue to finalize the Specific aim 1 and 2 objectives. The section of the SOW no longer funded is greyed out.**

   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	**1-6**	**X**	

• Catalytic bioscavenger will be purified using IMAC.			
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			
Specific Aim 3: Evaluate extracorporeal bioscavenging system in safety and feasibility model with healthy animals for 72 hours *in vivo* in swine.			
Major Task 4: Test administration of bioscavenger in ECLS system in swine			
Subtask 1- Write and obtain approval of animal research protocol by IRB and ACURO	**13-15**	**X**	
Subtask 2- Carry out randomized experiments in 3 groups (total n= up to 20) • Group 1 (n=6): 72-hour ICU experiments with animals on dialysis ECLS without bioscavenger. • Group 2 (n=6): 72-hour ICU experiments with animals on dialysis ECLS with bioscavenger. • Group 3 (n=6): 72-hour ICU experiments without ECLS (time controls).	**15-24**	**X**	
Milestone(s) Achieved: *In vivo* validation of ECLS+ bioscavenger prototype completed and ready to transfer to NHP model at USAMRICD			
Specific Aim 4: Evaluate extracorporeal bioscavenging system in combat relevant CWNA exposure in non-human primates with six-hour follow-up.			
Major Task 5: Test administration of ECLS + bioscavenger in NHPs exposed to CWNA			
Subtask 1 – Write and obtain approval of animal research protocol by IACUC and ACURO	**25-27**	**X**	**X**
Subtask 2 – Carry out randomized experiments in 2 groups (total n= up to 16). • Group 1 (n=8) will be surgically implanted with ECLS. Bioscavenger will be administered to dialysate. Twenty minutes later, the animals will be exposed to an otherwise lethal dose of CWNA and monitored for six hours for signs/symptoms of exposure. • Group 2 (n=8) will be surgically implanted with ECLS. Animals will be exposed to CWNA. Up to five minutes later, ECLS will be turned on, and bioscavenger will be administered to dialysate. The animals will be monitored for six hours for signs/symptoms of exposure.	**28-36**	**X**	**X**
Milestone(s) Achieved: Demonstrate use of novel ECLS bioscavenging system to promote protection against CWNA			

What was accomplished under these goals?

1. **A CRADA was established between ICD and AREVA to cover all aspects of performance of this project.**
2. **AREVA purchased the equipment, conducted training on it, and provided training to ICD.**
3. **AREVA assembled the testing platform.**

4. **AREVA began testing of the platform and performed initial proof of concept 3- hour testing of the ex-vivo circulation system with saline (n=3) followed up with further improvements in the testing platform and additional testing at 3 hours (n=3)**
5. **AREVA performed 6 hour circulation studies with donor swine blood (n=3) to work out initial technical details of blood viability, coagulation profile and metabolic marker sampling and analysis.**
6. **AREVA transferred the platform design to ICD and carried out troubleshooting conf calls with the ICD team to get them up to speed.**
7. **AREVA continued monthly-quarterly conf calls with ICD team providing technical support as ICD carried out their initial tests.**
8. **Upon report from ICD that there are technical difficulties with their system AREVA took part in redesigning the platform. The current configuration of the platform is provided in Figure 1.**

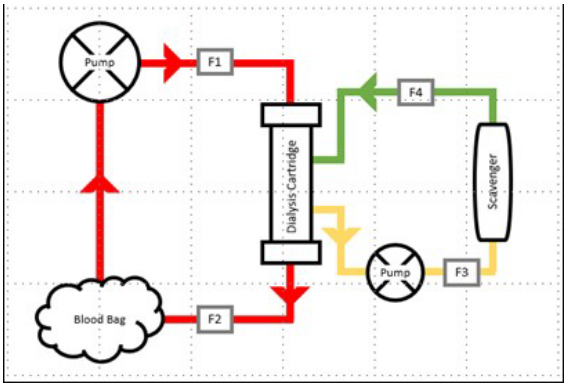

Figure 1: Schematic of ex-vivo circulation platform tested by AREVA. F1-F4 are blood sampling and measurement points (see technical description below).

Technical description:
An ex vivo circulation testing platform was established and undergoes testing by the expert team at AREVA. An NxStage hemodialysis membrane (NxStage Medical, Inc., Lawrence, MA) was used with a Masterflex peristaltic pump (Cole-Parmer, Vernon Hills, IL) to create a simple blood circulation loop (primary circuit). The blood reservoir is maintained at 37°C. The main circulation loop consists of 3/16 inch PVC tubing with sections of Tygon tubing for the peristaltic pump head. An infusion port is present on the post-NxStage membrane tubing. Clamps on ultrasonic flow meters (Transonic Systems Inc, Ithaca, NY) are placed post-pump and post NxStage membrane (Figure 1: F1, F2). A second dialysate loop was created using permeate/dialysate connections on the NxStage membrane (secondary circuit). 1/8 inch Tygon tubing was used for the secondary circuit with an infusion port added. Additional flow meters are placed after the pump (Figure 1: F3) and after the bioscavenger (Figure 1: F4). All flow meters operate in ¼ flow scale and will be zero-calibrated at the start of the study. Components of the circulation system were assembled using large bore connectors and quick release connectors to allow for ease of connection and handling during the experiment. The primary circuit NxStage membrane is be primed with normal saline along with most of the 3/16 tubing using the Masterflex pump and a standard 1-liter saline infusion bag. The secondary circuit is also flushed and primed.

Brief description of six-hour circulation studies to be continued with the remaining funding.
One liter of swine blood will be transferred into the blood bag. Priming will performed by connecting the blood bag pre-pump and having the post- membrane line waste out for disposal. In effect, the saline used for priming the membrane and circuit is removed from the system and will not dilute the swine blood. Once most of the saline is removed from the system, the circuit is returned to a closed loop and circulation can begin. RPM of the primary Masterflex pump will be adjusted to ensure primary circuit flow of 400 mL/min. The RPM of the secondary pump will be adjusted to ensure secondary circuit flow

of 50 mL/min. Baseline blood samples will be taken as soon as the circulating blood temperature reaches 37°C. Blood samples will be collected from both the primary circuit and secondary circuit at baseline and hourly thereafter through six hours. Minimum necessary blood volumes are drawn to ensure there is not a significant reduction in blood volume in the closed circulation system. Any leftover blood is returned to the circuit via the infusion port post-NxStage membrane. Blood gas analysis will be performed using a GEM4000 analyzer (ILWW, Bedford, MA), and plasma free hemoglobin will be measured by the spectrophotometric method using a SpectraMax microplate reader (Molecular Devices, San Jose, CA). Complete blood count (ADVIA 2120, Siemens; Munich, Germany) and basic coagulation panel e.g. PT, aPTT, activated clotting time [ACT]) (STAGO Compact Max, Diagnostica STAGO, Parsippany, NJ).

2. Products: List any products resulting from the project during the reporting period. If there are no products to report for the current quarter, state "Nothing to report."

- Virtual attendance to the ECMO and the Advanced Therapies for Respiratory Failure Symposium hosted by the Children's National Health System

3. Participants & Other Collaborating Organizations

What individuals have worked on the project?
Provide the following information for: (1) Project Directors (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).

Name:	Andriy Batchinsky, MD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	ORCID 0000-0001-8601-2827
Nearest person month worked:	1.0
Contribution to Project:	Overseeing conduct of the study, supervised study execution, data and sample collection and analysis, coordinating preparation of manuscripts and reports.

Name:	Teryn Roberts, PhD
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	ORCID 0000-0002-2460-6432
Nearest person month worked:	1.5
Contribution to Project:	Overseeing conduct of the study, supervising study execution, data and sample collection and analysis, experiment planning, report and manuscript preparations.

Name:	Jae Choi, PhD
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.1
Contribution to Project:	Overseeing conduct of the study, supervising study execution, data and sample collection and analysis, and coordinating the preparation of manuscripts and reports.

Name:	Brendan Beely
Project Role:	Research Coordinator
Researcher Identifier (e.g. ORCID ID):	0000-001-9442-9462

Nearest person month worked: 1.5
Contribution to Project: Assisting with study protocols and report preparation, routine laboratory procedures.

Name: Dan Wendorff
Project Role: Laboratory Manager
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.5
Contribution to Project: Animal protocol preparation, oversee lab technicians, routine laboratory procedures.

Name: Isabella Garcia
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.7
Contribution to Project: Assisting with large animal handling and protocol preparation

Name: Zachary Allen
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.6
Contribution to Project: Assisting with data collection and interpretation.

Name: Shubhneet Warar
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.8
Contribution to Project: Assisting with data collection and interpretation.

Name: Ryley Zapien
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.5
Contribution to Project: Assisting with data collection and interpretation.

Name: Cassandra Niemeyer
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.8
Contribution to Project: Assisting with data collection and interpretation.

Name: Yanyi Zang
Project Role: Postodctoral Fellow
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: .02
Contribution to Project: Assisting with ex vivo circulation study execution and post-circulation material analysis, biosample processing

Name: George Harea
Project Role: Research Associate II
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 4.2

Contribution to Project:	Assisting with ex vivo circulation study execution and post-circulation material analysis, biosample processing

Name:	John Jones
Project Role:	Statistician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.25
Contribution to Project:	Data analysis and interpretation.

Name:	Bridget Lee
Project Role:	Administrative Assistant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.7
Contribution to Project:	Assisting with the coordination of meetings with collaborator, meeting minutes, shipping samples.

4. **Changes/Problems:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

   a. Actual Problems or delays and actions to resolve them

   Provide a description of current problems or issues that may impede performance or progress of this project along with proposed corrective action. Also describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

   The progress on this project was most significantly affected by delays in initial contracting and delivery of funds to each party; delay in contractual negotiations; delay in establishing CRADA between AREVA and ICD; COVID-19 delays which affected the lab schedule and created a backlog of experiments on other projects which slowed down performance on this project. The latter is not a major concern as the POP ends in 2024 and we intend to fully execute the project by then and complete all ex-vivo experiments with donor pig blood.
   The remainder of the funds will be used to carry out the formal 6 hour ex vivo circulation studies with swine blood as originally proposed.

   b. Anticipated Problems/Issues

   We anticipated that the dialysis membrane initially proposed (nxStage) may not be functional for the specific fluid permeation rates required by ICD for their specific scavenger. Thus ICD continued with a different dialysis membrane while AREVA is developing a more universally used system usable with a variety of toxic factors.

5. **Special Reporting Requirements:**

   N/A