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TITLE: Portable NO Generation for Heparin-Free Extracorporeal Life Support in Combat Casualties

PRINCIPAL INVESTIGATOR: Andriy I. Batchinsky, MD

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14. ABSTRACT

The purpose of this project is to investigate a novel method to prevent coagulation disturbances during extracorporeal life support using nitric oxide (NO) gas. This approach will prevent local platelet aggregation and activation, eliminating the need for administration of systemic anticoagulant drugs that often cause untoward effects such as bleeding. Current NO gas delivery systems that are utilized for therapeutic inhalation of NO, such as during persistent pulmonary hypertension of newborns, are cumbersome, bulky and expensive – and are certainly not feasible for out-of-hospital application during prolonged field care. For this reason, in addition to investigating NO gas in a novel application as a local anticoagulant agent during ECLS, we will investigate a novel and portable/low impact NO gas delivery system that produces NO gas from air. The overarching **objective** of the study is to validate this portable NO generator for mitigation of coagulation disturbances during ECLS for multiorgan support in a combat-relevant trauma model with prolonged field care and aeromedical evacuation in large animals (swine). We **hypothesize** that mobile NO generation enables heparin free ECLS for lung and renal failure. This work will be accomplished by comprehensive *in vitro, ex vivo* and *in vivo* experiments identifying the safety and feasibility of portable NO generation, and then exhaustive testing of the system on both the bench and in animal studies.

15. SUBJECT TERMS

NONE LISTED

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1. INTRODUCTION:

This report serves as an annual report of activities and progress made by Dr. Andriy Batchinsky MD, Principal Investigator at AREVA/The Geneva Foundation and his team towards completion of work awarded to The Geneva Foundation as part of federal grant W81XWH-20-1-0833, titled "Portable NO Generation for Heparin Free Extracorporeal Life Support in Combat Casualties." The study is performed in collaboration with Dr. Binglan Yu at Massachusetts General Hospital. The purpose of this project is to investigate a novel method to prevent bleeding and thrombotic complications during extracorporeal life support using nitric oxide (NO) gas. This approach will prevent local platelet aggregation and activation, eliminating the need for administration of systemic anticoagulant drugs that often cause untoward effects. Current NO gas delivery systems that are utilized for therapeutic inhalation of NO, such as during persistent pulmonary hypertension of newborns, are cumbersome, bulky and expensive – and are certainly not feasible for out-of-hospital application during prolonged field care. For this reason, in addition to investigating NO gas in a novel application as a local anticoagulant agent during ECLS, we will investigate a novel and portable/low impact NO gas delivery system that produces NO gas from air. The overarching **objective** of the study is to validate this portable NO generator for mitigation of coagulation disturbances during ECLS for multiorgan support in a combat-relevant trauma model with prolonged field care and aeromedical evacuation in large animals (swine). We **hypothesize** that mobile NO generation enables heparin free ECLS for lung and renal failure. This work will be accomplished by comprehensive *in vitro* and *in vivo* experiments identifying the safety and feasibility of portable NO generation; and then exhaustive testing of the system on both the bench and in animal studies.

2. KEYWORDS:

Extracorporeal life support, multiorgan failure, nitric oxide, prolonged field care, combat trauma, anticoagulation, bleeding, thrombosis, respiratory therapy, hemostasis, ECMO

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Highlighted objective percentages denote updates from Y1 Annual Report.

Major Task 1: Obtain regulatory approval for laboratory/non-animal related studies.	Months	Site 1 - AREVA	Site 2 - MGH	Percentage Complete (%)	Status:
Objective 1.1: Draft laboratory protocols for ex vivo work; submit for internal approvals	1-3	Batchinsky	Yu	100%	Completed Y1Q1
Milestone 1: Obtain local approvals for laboratory protocols	3	Batchinsky	Yu	100%	Completed Y1Q1
Major Task 2: Measure and characterize particle release, secondary nitrogen species generation during NO generation; and establish safety controls/backup generation system					
Objective 2.1: Identify and quantify secondary gas generation (ex. N_2O_2, N_2O_4, etc.) during NO generation	1-12		Yu	100%	Completed 29 Sept 21
Objective 2.2: Investigate filtration capacity of in-line HEPA filter during NO generation	1-12		Yu	100%	Completed Y2Q2

and determine duration of use over 10 days					
Objective 2.3: Develop failsafe system to disable NO production if post-scavenging NO level exceeds established upper threshold of NO production	1-12		Yu	100%	Completed 29 Sept 21
Objective 2.4: Develop secondary backup system to be initiated if NO production fails/low NO threshold limit detected	1-12		Yu	100%	Completed 29 Sept 21
Objective 2.5: Conduct safety testing of portable NO generation equipment during hypobaric conditions	1-12	Batchinsky/ Chauvin		90%	Further discussion with Wyle team in next quarter
Objective 2.6: Assess NO exposure potential to chamber operators while using portable NO generation equipment during hypobaric conditions	1-12	Batchinsky/ Chauvin		90%	Further discussion with Wyle team in next quarter
Milestone 2: NO generation system optimized for use with ECLS sweep gas	12	Batchinsky	Yu		
Specific Aim 2: Assess feasibility and safety of NO generation in conjunction with ECLS during 24 hours ECLS in healthy swine					
Major Task 3: Configure ECLS circuit *ex vivo* to incorporate NO generation system.					
Objective 3.1: Construct *ex vivo* test circuit with NO generator optimally connected to membrane oxygenator	6-12	Batchinsky		100%	Completed 29 Sept 21
Objective 3.2: Validate flows and pressures in *ex vivo* test circuit with 0.9% Normal Saline and glycerol/water mix (blood analog) to determine optimal generator settings to reach NO target concentrations	6-12	Batchinsky		100%	Completed 29 Sept 21
Milestone 3: NO Generator optimally integrated/connected to ECLS System.	12	Batchinsky		100%	
Major Task 4: Conduct *in vivo* testing of NO gas generation and delivery during ECLS in healthy swine for 24 hours circulation [n=4pigs/group + 2 replacements = 10 pigs total]					
Objective 4.1: Draft animal use protocol for 24-hour animal study, submit for approvals.	6-12	Batchinsky		100%	Completed Y2Q1
Milestone 4: Obtain local regulatory approval for 24-hour *in vivo* study	9	Batchinsky		100%	Completed 01 Oct 2021
Milestone 5: Obtain secondary level ACURO approval for 24-hour *in vivo* animal protocol	12	Batchinsky		100%	Completed Y2Q1
Objective 4.2: Assess platelet activation and consumption during 24 hours ECLS *in vivo* in healthy swine without systemic heparinization comparing NO gas administration into membrane oxygenator for regional anticoagulation (n=4) versus controls (n=4)	12-18	Batchinsky		100%	Completed Y3, manuscript preparation in progress
Objective 4.3: Assess systemic coagulation status during 24 hours ECLS *in vivo* with and without administration of NO gas into membrane oxygenators.	12-18	Batchinsky		100%	Completed Y3, manuscript preparation in progress

Objective 4.4: Assess NO delivery during 24 hours ECLS *in vivo* with and without administration of NO gas into membrane oxygenators using NO sensors and measurement of NO metabolites (nitrate/nitrite) and methemoglobin in circulating blood.	12-18	Batchinsky	Yu	100%	Completed Y3, manuscript preparation in progress
Objective 4.5: Assess circuit patency, pressures, and blood flow during 24 hours ECLS in healthy swine with and without NO gas administration into membrane oxygenators.	12-18	Batchinsky		100%	Completed Y3, manuscript preparation in progress
Objective 4.6: Assess thrombus deposition on explanted ECLS circuits following 24-hours ECLS *in vivo* in healthy swine with and without NO gas administration.	12-18	Batchinsky		100%	Completed Y3, manuscript preparation in progress
Milestone 6: Establish optimal NO generation and delivery settings to enable heparin-free ECLS and attenuate platelet activation	18	Batchinsky	Yu		
Specific Aim 3: Test the NO generator for feasibility and safety of use in large animal model of combat-relevant trauma treated with ECLS					
Major Task 5: Conduct in vivo testing of NO gas generation and delivery during ECLS in a combat-relevant polytrauma model in swine [8 pigs x 2 groups = 16 animals]					
Objective 5.1: Draft animal use protocol for 72-hour animal study, submit for approvals.	6-12	Dr. Batchinsky		100%	
Milestone 7: Obtain local regulatory approval for 72-hour polytrauma study	9		Dr. Batchinsky	100%	
Milestone 8: Obtain secondary level ACURO approval for polytrauma with 72-hour follow-up animal protocol	12		Dr. Batchinsky	100%	
Objective 5.2: Perform 72 hr study of polytrauma treated with ECLS comparing standard heparin anticoagulation during circulation (Group 1) versus local NO anticoagulation using the NO generation system and infusion into the ECLS sweep gas (Group 2)	18-36		Dr. Batchinsky	5%	
Objective 5.2.1: During last 6 hours of large animal studies in Task 5, conduct simulated aeromedical evacuation at 5,000 ft., 8,000 ft., 30,000 ft. simulated altitude	18-36	Batchinsky/ Chauvin		0%	
Objective 5.3: Evaluate platelet activation and consumption, coagulation profile and hematology in Group 1 versus Group 2	18-36	Batchinsky		0%	
Objective 5.4: Evaluate systemic effects of NO administration, including vascular resistance, methemoglobin fraction and NO metabolite generation during 72 hours ECLS following polytrauma.	18-36	Batchinsky		0%	
Objective 5.5: Evaluate thrombus deposition and circuit patency following 72 hrs ECLS in	26-36	Batchinsky		0%	

Group 1 and Group 2 using scanning electron microscopy and digital imaging at the end of circulation.					
Objective 5.6: Assess bleeding and thrombotic complications/risks in Group 1 versus Group 2	26-36	Batchinsky		0%	
Objective 5.7: Assess inflammatory mediators, injury markers and histopathological evidence in Group 1 versus Group 2	26-36	Batchinsky		0%	
Milestone 9: Determine the safety and efficacy of NO-gas generation for anticoagulation during ECLS in polytrauma.	36	Batchinsky		0%	
Objective 6.1: Finalize sample and data analysis	34-36	Batchinsky		0%	
Objective 6.2: Draft final progress reports and manuscripts	34-36	Batchinsky		0%	
Milestone 10: Completion of final reports and manuscripts	36	Batchinsky		0%	

What was accomplished under these goals?

-Phase 1, Specific Aim 1: Evaluate the purity and safety of electrically generated NO for infusion into ECLS membranes via the sweep gas.

1) **Major Activity 1: Obtain regulatory approval for laboratory studies**
   a) **Objective 1.1:** Draft laboratory protocols for laboratory/non-animal studies; submit for internal approvals
      - **Major activities:** Completed
      - **Results/developments/achievements:** Completed Y1Q1.
2) **Major Activity 2: Measures and characterize particle release, secondary nitrogen species generation during NO generation; and establish safety controls/backup generation system.**
   a) **Objective 2.1-2.5:** Develop generator and backup system, conduct safety testing, identify and quantify secondar gas generation, investigate filtration capacity of in-line HEPA filter for use over 10 days.
      - **Major activities:**
      - AREVA team observed that during animal experiments, there was a trans-generator decrease in fraction of oxygen delivered into the membrane oxygenator from the generator inlet to the outlet. We had concern that the oxygen flow chamber may have a leak. The generator was shipped to MGH to assess. MGH evaluated the leaking problem during NO delivery with a range of oxygen gas flow rates from 1-10 L/min (see results in Table 1). No flow chamber leaks were noted, and the decrease in oxygen content at the generator outlet was attributed to mixing of the oxygen gas with NO and air (carrier gas for the NO). To increase the oxygen concentration in the sweep gas, increasing oxygen flow in the gas mixture is advised. The generator was shipped back to AREVA after evaluation, but the outer housing encountered damage during shipping. The AREVA team attempted to calibrate the generator sensors to assess if damage occurred. There appeared to be an issue with the power supply and alarm system. AREVA notified MGH. MGH advised that AREVA contact Odic Inc. (contracting organization that

assembles the generator). Odic team provided troubleshooting instructions. AREVA followed the troubleshooting instructions and generator function was resolved. A power cable disconnect had occurred in shipping and was causing the observed issues. AREVA has performed repeat calibration and test runs and are now ready to initiate 72-hour animal studies with the generator.

Table 1. Results for evaluation of potential NO generator oxygen flow chamber leak.

Gas Flow (L/min)			FiO_2 (%)	
O_2 inlet	NO chamber	Gas outlet (O_2 + gas from NO chamber)	Measured	Expected
1.036	0.64	1.68	64.5	69.7
2.030	0.81	2.84	77.6	77.6
4.030	0.90	4.93	86.5	85.5
6.060	1.03	7.09	90.8	88.6
8.036	0.93	8.96	93.7	91.8
10.042	0.57	10.62	96.1	95.7

b) **Objective 2.6:** Assess NO exposure potential to chamber operators while using portable NO generation equipment during hypobaric conditions.
- **Major activities:**
- Wyle flight team advised that introduction of NO into the hypobaric chamber would require installation of inlet/outlet hypobaric chamber gas channels to minimize NO accumulation within the chamber during the flight simulation; and to address pressure differentials that would affect delivery. Feasibility of this approach is now being assessed.
- **Results/developments/achievements:** In progress, to be completed in the next reporting period.

<u>-Phase 1, Specific Aim 2: Assess feasibility and safety of NO generation in conjunction with ECLS during 24 hours ECLS in healthy swine.</u>

3) Major Activity 3: Configure ECLS circuit ex vivo to incorporate NO generation system.
- **Major activities:** Completed – see Y1 annual report
- **Results/developments/achievements:** Completed – see Y1 annual report

4) Major Activity 4: Conduct in vivo testing of NO gas generation and delivery during ECLS in healthy swine for 24 hours circulation (n=4 pigs/group + 2 replacements)

a) **Objective 4.1:** Draft animal use protocol for 24-hour animal study, submit for approval.
- **Major activities:** Completed.
- **Results/developments/achievements:** Objective completed in Y2Q1.

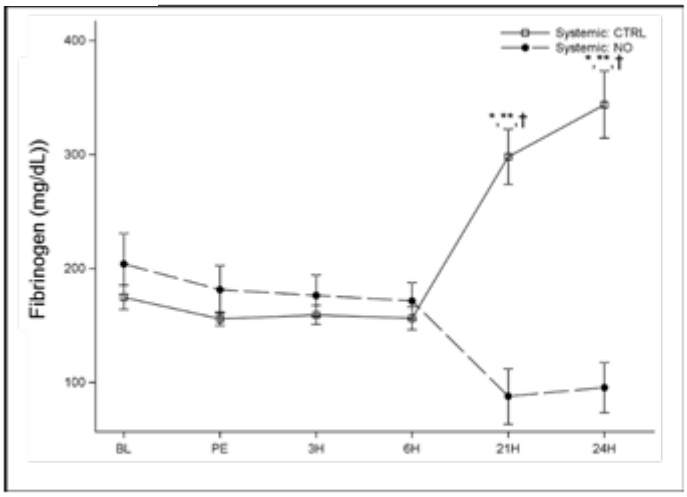
b) **Objective 4.2:** Assess platelet activation and consumption for 24 hours ECLS *in vivo* in healthy swine without systemic anticoagulation comparing NO gas administration into membrane oxygenator for regional anticoagulation (n=4) versus controls/no NO gas (n=4)
- **Major activities:**

- Animal studies completed. Manuscript summarizing animal studies in progress.
- **Results/developments/achievements:**
- No group difference in platelet count or surface activation markers were observed, as detailed in the Y3Q2 report. Collagen stimulated platelet aggregation response was reduced in the NO group vs CTRL at 24-hours, and clot strength trended lower in NO versus CTRL from 21-24 hours – suggesting a potential functional difference in platelet activation/aggregation. This will be further investigated in the 72-hour studies. We also anticipate greater between-group differences in the 72-hour studies which involve an injury component, as well as longer duration of extracorporeal circulation. Membrane thrombus area was significantly lower in the NO group (more details below), which suggests reduced platelet consumption in the extracorporeal circuit.

c) **Objective 4.**3: Assess systemic coagulation status for 24 hours ECLS in vivo with and without administration of NO gas into membrane oxygenators

- **Major activities:**
- Analyses completed and manuscript preparation in progress.
- **Results/developments/achievements:**
- The most outstanding difference in coagulation between groups was the finding that fibrinogen concentration became significantly elevated by 24-hours in CTRL, which did not occur in the NO group. We hypothesize that NO may prevent or suppress systemic inflammation that occurs in response to extracorporeal circulation and can yield elevated fibrinogen levels. This will be further evaluated in the 72-hour studies.

Figure 1



*Significant within CTRL versus BL, **Significant within NO versus BL, †Significant CTRL vs NO. Alpha=0.05 for significance.

d) **Objective 4.**4: Assess NO delivery for 24 hours ECLS in vivo with and without administration of NO gas into membrane oxygenators using NO sensors and measurement of NO metabolites (nitrate/nitrite) and methemoglobin in circulating blood.

- **Major activities:**

- MGH evaluated NO metabolite concentrations and did not detect a significant difference between groups (Figure 2). Methemoglobin remained at normal levels (<2%) at all times in both groups.
- **Results/developments/achievements:**

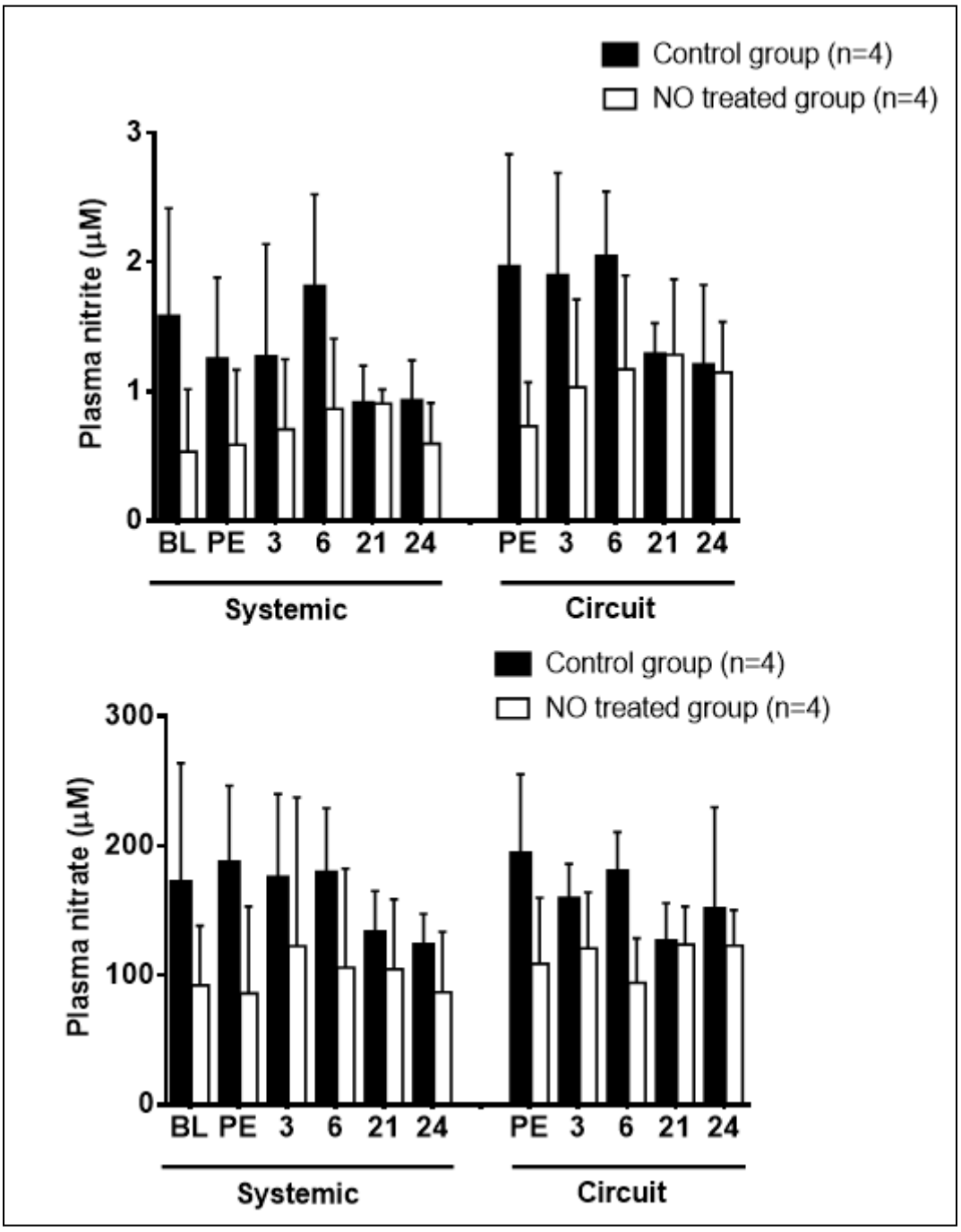


Figure 2. Concentration of nitric oxide (NO) metabolites nitrate and nitrite in plasma from swine that received NO gas during extracorporeal circulation (NO, n=4) versus CTRL group (n=4).

- This study demonstrated the safety profile of NO gas administration via extracorporeal membrane oxygenator sweep gas for 24 hours across a range of concentrations. These results are now in preparation for publication.

e) **Objective 4.**5: Assess circuit patency, pressures, and blood flow for 24 hours ECLS in healthy swine with and without NO gas administration into membrane oxygenators.
   - **Major activities:**
   - Animal studies completed. No group differences in circuit patency, pressures, or extracorporeal blood flow observed. All circuits remained patent.
   - **Results/developments/achievements:**
   - NO gas delivery did not affect extracorporeal circuit pressures or flow rates. We will continue to assess this in the 72-hour phase of the study. Manuscript summarizing 24-hour study is in progress.

f) **Objective 4.**6: Assess thrombus deposition on explanted ECLS circuits following 24-hours ECLS in vivo in healthy swine with and without NO gas administration.
   - **Major activities:**
   - We completed oxygenator dissection, imaging, and image analysis for all oxygenators (n=8) from the 24-hour study.
   - **Results/developments/achievements:**
   - Thrombus area was significantly lower (p=0.0073) in the NO group (10.6 ± 0.9% thrombus area coverage) compared to CTRL (15.6 ± 1.2% thrombus area). Results in preparation for publication. This is an exciting finding that will be further assessed in the 72-hour study phase.

-Phase 2, Specific Aim 3: Test the eNO generator for feasibility and safety of use in large animal model of combat-relevant trauma treated with ECLS.

5) **Major Activity 5: Conduct in vivo testing of NO gas generation and delivery during ECLS in a combat-relevant polytrauma model in swine for 72 hours [8 pigs x 2 groups = 16 animals]**

a) **Objective 5.1:** Draft animal use protocol for 72-hour animal study, submit for approval
   - **Major activities:** The AREVA/Geneva team drafted the animal protocol (an amendment to the previous protocol – per request of the UTSA IACUC). The protocol amendment was submitted for IACUC approval. IACUC approval was received on 14 Mar 23. ACCURO review/approval was completed and received on 01 May 23. Protocols are now in place and the team is trained so that the studies can begin. The team was granted a 1 year no-cost extension for this project, during which the 72-hour animal studies will be completed.
   - **Results/developments/achievements:** Task completed.

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

Through this project, Dr. Yu is mentoring several postdocs at MGH (Drs. Stefano Gianni, Dario Winterton, Stefano Cenci, Talisa Buehl) and one respiratory staff member (Hatus V. Wanderley) with one-on-one training and testing of the eNO device at MGH.

At AREVA, medical school students from University of the Incarnate Word School of Osteopathic Medicine (UIW SOM) are receiving training through this project as they begin research rotations and internships with the AREVA team. Dr. Batchinsky has an appointment as Director of Department of Translational Medicine at UIW SOM, and has opened the laboratory to provide one-of-a-kind training and mentorship for the medical students. These students are learning essential research skills and gaining real-life experience in intensive care patient management in the animal ICU. For example, students learn to adjust the ECLS system settings and also learn to operate the eNO generator through the ex vivo circulation testing and 24-hour animal studies. The students are trained on ICU skills, anesthesia, vital signs recording and monitoring, blood gas collection and analysis, and coagulation tests during the animal studies. The students are also learning to write scientific abstracts for international research conferences, and some were invited to present work performed at the AREVA lab at these meetings.

How were the results disseminated to communities of interest?
Dr. Batchinsky presented the project and demonstrated the benchtop prototype NO gas generator at a San Antonio site visit from US Army MRMC TATRC Office.

The results were disseminated at key medical conferences including the 2023 Military Health System Research Symposium. Drs. Batchinsky and Roberts also presented this work in a site visit by The Geneva Foundation Board of Directors, at which the board expressed great enthusiasm. The AREVA team also shared this work with Emergency Medicine Physician Dr. Marinaro from University of New Mexico Hospital who expressed interest in this heparin-free approach for pre-hospital ECMO and extracorporeal cardiopulmonary resuscitation applications – inspiring future research efforts. Drs. Batchinsky and Roberts were invited to contribute to the Extracorporeal Life Support Organization clinical guide, the ELSO "Red Book" 6th edition, to the chapter on biomaterials and alternative coagulation management strategies.

Dr. Yu demonstrated the newly built portable eNO device to Dr. Lorenzo Berra (Medical Director of Respiratory Care) and Dr. Harris Stuart (Division Chief, Wilderness and Emergency Medicine) at MGH. Collaborating with Drs. Berra and Stuart, we submitted an NIH grant proposal of studying high dose NO as an antimicrobial to treat patients with acute bacterial pneumonia. Recently, our group has been approached by a physician, Dr. Laura Mercado at Beth Israel Deaconess Medical Center, Harvard Medical School. Dr. Mercado originally came from Sucre (capital of Bolivia), a relatively high-altitude city. A potential collaboration of testing the electrically generated NO from our device in pregnant patients with respiratory failure at high altitude is in progress.

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

In the next reporting period, AREVA/MGH plan to finalize a manuscript draft summarizing the 24-hour animal studies to be submitted for peer review and publication. AREVA will initiate the 72-hour animal studies, and plasma samples will be sent to MGH for NO metabolite assessment as the studies are completed.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
Development of a solution for coagulation disturbance during ECLS that does not involve systemic anticoagulation would radically improve the safety of this life-saving therapy, and would also make ECLS available to severely wounded with hemorrhagic complications who otherwise could not receive therapy due to anticoagulant contraindications.

Pending results of this study, this newly built, lightweight, and portable electric nitric oxide (eNO) generator will make it possible for ambulatory applications such as in the remote areas, emergency rescue in helicopter, or battlefields. Furthermore, this economical eNO device will enable increased accessibility to NO treatment, including for patients with chronic lung diseases in developing countries.

What was the impact on other disciplines?
Use of ECLS for applications outside of combat trauma is increasing, and all forms of ECLS could benefit from a circuit-focused/non-systemic solution to manage coagulation complications. For example, ECLS systems have been utilized during the COVID-19 pandemic to treat COVID pneumonia. ECLS is also used for pediatric and neonatal conditions where therapeutic anticoagulation administration is challenged by developmental differences in coagulation between neonates/pediatrics and adults. This approach of NO gas administration could also be applied to cardiopulmonary bypass systems.

The current eNO device can generate high dose NO over 100 ppm. NO, at higher doses (> 80 ppm) has antimicrobial activity against bacteria, fungi, helminths, protozoa, and viruses.[1] Accumulating *in vitro and in vivo* evidence supports the use of high dose NO, a potent free radical and nitrosating agent, as an antimicrobial.[2-10] Recently, we reported that intermittent breathing high dose NO (up to 300 ppm) reduced bacterial counts in the lung and spleen and improved overall survival of mice in a murine model of *Klebsiella pneumonia*.[4] In a case report, we showed safety and clinical benefits of delivering high dose NO (160 ppm) to a teenaged cystic fibrosis patient with *Burkholderia cepacia*.[6] With the challenges presented by the novel coronavirus causing COVID-19, we received compassionate use approval to test high dose NO therapy (160 ppm, 30 min, twice/day), which was well tolerated and could be an effective adjuvant rescue therapy for patients with severe COVID-19.[11] Similarly, we recently reported a case series of six spontaneously breathing pregnant patients with severe COVID-19 who were treated with high-dose iNO (160–200 ppm) delivered by mask for 30 minutes, twice daily for up to 14 days. We found that breathing a high dose of NO was associated with improved oxygenation and reduced respiratory rate for pregnant patients with severe or critical COVID-19, resulting in deliveries of healthy newborns in all six patients.[5] From a retrospective cohort study, we reported that in twenty pregnant hospitalized women with severe/critical bilateral x-ray proven pneumonia, treatment with high-dose iNO (200 ppm, 30 min, twice daily) decreased oxygen supplements and shortened the length of stay in ICU and hospital, compared with fifty-one patients who received standard of care alone.[12] No adverse events were observed in the twenty patients treated with high-dose iNO. Preliminary results from our recent randomized trial demonstrated that inhaled NO cleared viral counts in the blood and sputum of severe COVID-19 patients faster than control. It is conceivable to hypothesize that intermittent inhalation of high dose NO generated from the newly developed, portable NO device is a safe and effective antimicrobial therapy for treating patients with respiratory tract infections. Thus, a lightweight, portable, economical NO generator will increase the accessibility to NO therapy in

hospital settings, remote areas with scarce medical resources, and for outpatient and home settings for chronic cardiovascular and pulmonary diseases.

What was the impact on technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Development of a portable NO generator that produces NO from air (versus extremely expensive NO gas tanks and delivery systems) could be an affordable option for treatment of pulmonary hypertension in newborns in developing countries where this therapy is not readily available.

5. CHANGES/PROBLEMS:

Nothing to report

Changes in approach and reasons for change

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report.

Changes that had a significant impact on expenditures
Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report.

Significant changes in use or care of human subjects
Nothing to report.

Significant changes in use or care of vertebrate animals
Nothing to report.

Significant changes in use of biohazards and/or select agents
Nothing to report.

6. PRODUCTS:

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

Journal publications.
Yu, B; Wanderley, HA; Gianni, S; Carroll, RW; Ichinose F; Zapol, WM; Berra, L. Development of nitric oxide generators to produce high-dose nitric oxide for inhalation therapy. *Nitric oxide.* 2023; 138-139: 17-25.

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

Ryerson LM, Annich G, Batchinsky AI, Martucci G, Roberts TR, Thiagarajan R, Vandebriele C, MacLaren G. "Adverse effects of extracorporeal life support" in *Extracorporeal Life Support: The ELSO Red Book 6th Edition.* 2022.

Other publications, conference papers and presentations.

Podium presentation: Roberts TR, Zang Y, Wendorff DS, Harea G, Beely BM, Allen ZH, Garcia I, Niemeyer C, Warar S, Zapien R, Wick T, Mohnike M, Selkow A, Melvin A, Reynolds MM, Batchinsky AI. Evaluation of a nitric oxide eluting extracorporeal circuit and NO gas-based heparin-free anticoagulation: A 72-hour ICU study in healthy swine. *39th Annual Children's National Symposium: ECMO & the Advanced Therapies for Cardiovascular and Respiratory Failure.* 26 Feb-01 Mar 2023. Keystone, CO, USA. ***abstract presented in "Best Scientific Abstract Competition" session.***

Podium presentation: Roberts TR, Zang Y, Yu B, Wendorff D, Beely B, Harea G, Persello A, Berra L, Zapol W, Batchinsky AI. Electrically-generated nitric oxide gas for circuit-centered anticoagulation during 24-hours heparin-free artificial lung support. *Military Health System Research Symposium 2023.* 2023. Kississimmee, FL, USA.

- **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**

  MGH has built a portable eNO generator with a complete alarm and backup system.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Andriy Batchinsky, MD
Project Role:	Principal Investigator
Researcher Identifier:	0000-0001-8601-2827
Nearest Person Month Worked:	0.9
Contribution to Project:	Design, overesight and carrying out the project protocol, collecting and analyzing data, preparing and finalizing manuscripts and reports.

Name:	Binglan Yu, PhD

Project Role:	Co-Principal Investigator
Researcher Identifier:	0000-0001-5496-2131
Nearest Person Month Worked:	1.2
Contribution to Project:	Directly guide and participate in all MGH experiments.

Name:	Teryn Roberts, PhD
Project Role:	Co-Investigator
Researcher Identifier:	0000-0002-2460-6432
Nearest Person Month Worked:	0.8
Contribution to Project:	Design and oversight of the project protocol, collecting and analyzing data, preparing and finalizing manuscripts and reports.

Name:	Brendan Beely
Project Role:	Research Coordinator
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.5
Contribution to Project:	Execution of animal study, preparation of protocols and reports.

Name:	Jacob Callahan
Project Role:	Laboratory Technician
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.7
Contribution to Project:	Assisting with data collection and technical procedures. Assisting with animal studies..

Name:	Brittany Ogozaly
Project Role:	Laboratory Technician
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.6
Contribution to Project:	Assisting with data collection and technical procedures. Assisting with animal studies..

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report.

What other organizations were involved as partners?
N/A

8. **SPECIAL REPORTING REQUIREMENTS: N/A**
9. **COLLABORATIVE AWARDS:** *N/A*

This is a duplicative report with tasks marked with the responsible PI and research site.

QUAD CHARTS: *N/A*

See attached.

10. APPENDICES:

N/A