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

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CONTRACTING ORGANIZATION: The Geneva Foundation, Autonomous Reanimation and Evacuation Research Program (AREVA), San Antonio, TX

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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?

Note – below is the revised and approved SOW

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	75%	In process, to be completed

and determine duration of use over 10 days					next quarter
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		10%	Awaiting delivery of generator from MGH to AREVA
Objective 2.6: Assess NO exposure potential to chamber operators while using portable NO generation equipment during hypobaric conditions	1-12	Batchinsky/ Chauvin		10%	Awaiting delivery of generator from MGH to AREVA
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		90%	IACUC approved, under review by ACURO
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		75%	Protocol submitted to ACURO, under review
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		5%	Awaiting ACURO approval to start animal work, team trained on procedures
Objective 4.3: Assess systemic coagulation status during 24 hours ECLS *in vivo* with and	12-18	Batchinsky		5%	Awaiting ACURO

without administration of NO gas into membrane oxygenators.					approval to start animal work, team trained on procedures
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	5%	Awaiting ACURO approval to start animal work, team trained on procedures
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		5%	Awaiting ACURO approval to start animal work, team trained on procedures
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		5%	Awaiting ACURO approval to start animal work, team trained on procedures
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		0%	
Milestone 7: Obtain local regulatory approval for 72-hour polytrauma study	9		Dr. Batchinsky	0%	
Milestone 8: Obtain secondary level ACURO approval for polytrauma with 72-hour follow-up animal protocol	12		Dr. Batchinsky	0%	
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	0%	
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	18-36	Batchinsky		0%	

and consumption, coagulation profile and hematology in Group 1 versus Group 2					
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 Group 1 and Group 2 using scanning electron microscopy and digital imaging at the end of circulation.	26-36	Batchinsky		0%	
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:**
      - Protocol for laboratory testing of new generator drafted and internally approved at MGH and AREVA.
      - **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:**

- MGH completed building a new portable electric nitric oxide (eNO) generator. Its dimension is L×W×H=13 in×8.5 in×3.5in, and weighs 5.8 lb (see photos Figure 1).

Figure 1. New portable electric nitric oxide generator.

- MGH tested and validated the newly built eNO generator in a test model, which included measuring NO and NO_2 levels under different O_2 flow rates, duty cycles, pulse rates, and fan efficiency (see results Table 1). Table 1 results will serve as a reference for future in vitro and in vivo experiments. The selected flow rates were selected based on recommendation from Dr. Batchinsky as to what sweep gas flow rates would be needed for ECLS application.
- MGH completed a failsafe system including an alarm to alert operators when NO and NO2 exceed desired levels, a LED signal when starting the NO generation, and an LED indicator for battery life. Table 2 includes battery life measured at various duty cycles.
- MGH completed a secondary backup system including a spare set of NO electrodes, power supply, transformers, electric boards, etc.
- MGH and AREVA had a meeting to discuss progress on testing and transfer of the eNO generator to AREVA, the expected timelines and detailed plans to test the new generator in a healthy swine model at AREVA.

Table 1. Nitric oxide (NO) and nitrogen dioxide (NO_2) levels measured under various oxygen (O_2) flow rates. eNO generator settings: flow rate: 0.5 L/min, fan: 40, Pulse period: 500 ms

Diluted with 99.9% O_2 (L/min)	1		2		3		4		5		6		7		8	
Duty cycle (%)	5	12	8	18	11	25	13	30	15	35	17	45	20	60	25	65
NO (ppm)	80	200	80	200	80	200	80	200	80	200	80	200	80	200	80	200
NO_2 (ppm, without scavenger)	1.1	3.6	1.0	3.5	1.1	3.9	1.1	4.0	1.0	4.0	1.0	4.0	1.0	4.5	1.1	3.9
NO_2 (ppm, with a scavenger)	0.3	1.8	0.4	2.1	0.4	2.3	0.4	2.2	0.4	2.3	0.4	2.7	0.5	3.0	0.5	2.5

Table 2. NO, NO_2 levels, and battery life measured at various duty cycles.

eNO flow rate (L/min)	0.5	0.5	0.5
Diluted with 99.9% O_2 (L/min)	5	5	8
Duty cycle (%)	35	50	35
Fan	30	40	50
NO (ppm)	175-180	250	115-120
NO_2 (ppm, without scavenger)	2.5	4.5	1.6
NO_2 ppm, with a scavenger)	1.6	1.7	0.8
Battery life (hr)	—	—	3 hr 10 min

- **Results/outcomes/achievements:**
- Achievement: new portable eNO generator designed and constructed.
- Benchtop validation of eNO generator for ECLS applications completed.
- In the next reporting period, MGH expects to finish testing the purity of the electrically generated NO with ICP-MS; and the efficiency of using scavenging-HEPA filtration system to remove all contaminations (NO derivatives and metal particles) in the electric NO.
- In the next reporting period, MGH plans to transfer the portable eNO generator to AREVA for in vivo testing.

b) **Objective 2.6:** Assess NO exposure potential to chamber operators while using portable NO generation equipment during hypobaric conditions

- **Major activities:**
- This objective will be performed at AREVA following transfer of eNO generator from MGH.
- **Results/developments/achievements:** Not initiated.

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

3) Major Activity 3: Configure ECLS circuit ex vivo to incorporate NO generation system.

a) **Objective 3.1 and 3.2:** Construct ex vivo test circuit with NO generator optimally connected to membrane oxygenator. Validate flows and pressures in ex vivo test circuit with blood analog to determine optimal generator settings to reach NO target concentrations.

- **Major activities:**
- The AREVA team conducted several studies demonstrating the feasibility of incorporating a prototype benchtop laboratory NO gas generator (non-portable) into two different ECLS systems – the CardioHELP (Maquet/Getinge) and the XLUNG System (Xenios/Fresenius). First, saline was circulated through the system and NO gas was delivered into the oxygenator sweep gas at a range of 40-120 ppm. The generator settings required to deliver NO gas at the target concentration were recorded. The oxygenator sweep gas flow rate and the circuit blood flow rates were adjusted, and the generator settings required to achieve the target NO dose at each setting was determined. The concentration of NO gas at the membrane gas inlet and outlet were recorded. This entire process was repeated using injured swine blood collected from a separate study/project where the method of euthanasia was exsanguination.
- **Results/developments/achievements:**
- **Figure 2** shows the benchtop test setup incorporating the prototype laboratory NO generator with an ECLS system with swine donor blood circulating through the system.
- **Table 3** is an example of eNO generator settings require to achieve target NO concentrations of 40, 80 and 120 ppm at the membrane oxygenator inlet using two different ECLS systems. Note that at high sweep gas concentrations, the higher NO target concentrations could not be achieved with maximum NO generator settings using the prototype generator. The new portable generator that is being developed at MGH for this project will have a greater NO output capacity to address this.
- When testing the system with saline, the NO gas concentration delivery at the membrane inlet and the NO concentration measured at the membrane outlet were similar. When testing the system with blood, the NO gas concentration measured at the outlet was significantly lower than the NO gas concentration delivered at the inlet, indicating transfer of NO into the blood.
- Delivery of NO gas into the membrane oxygenator sweep gas had no effect on the pressure readings within the ECLS circuit. This was validated with both saline and blood.

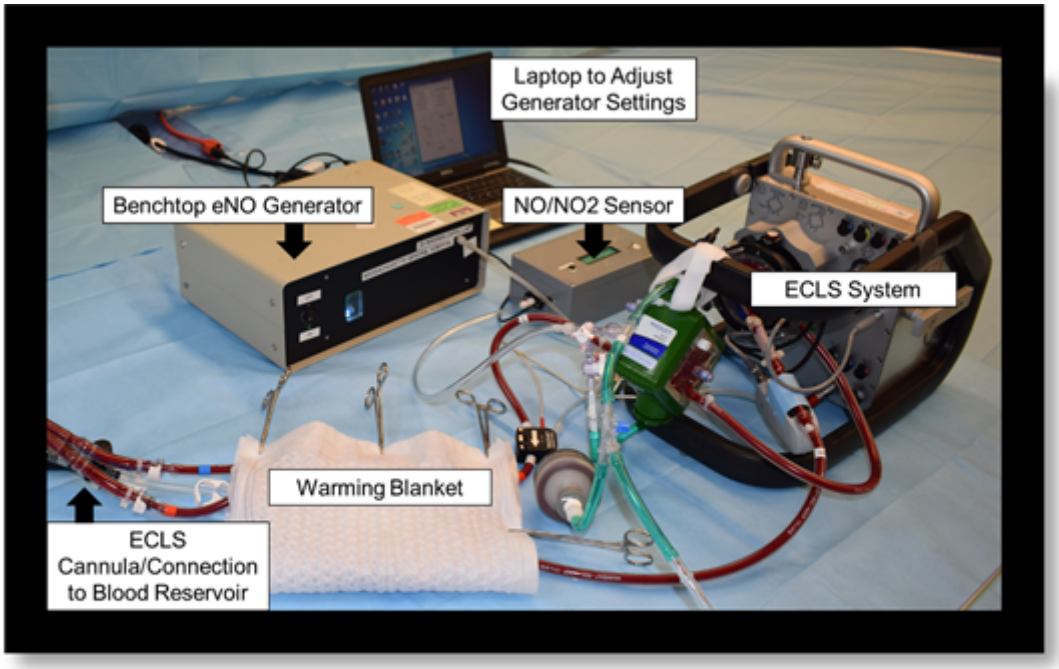

Figure 2. Photograph of old/prototype benchtop laboratory electric nitric oxide generator prototype connected to an ECLS System (CardioHelp System) for assessment of NO gas delivery into membrane oxygenator under various sweep gas and blood flow settings at AREVA.

Table 3. Example eNO generator settings required to achieve target NO concentration delivery into membrane oxygenator inlet using two different ECLS systems. "DUTY" is the generator command/adjustment that controls the number of pulses/sparks delivered per minute and the delay and duration of each spark to achieve the target NO dose. The DUTY settings range from 0-100 where 100 represents maximum NO output.

XLUNG ECLS SYSTEM :				
Sweep Gas (L)	Blood Flow (L)	DUTY (40 ppm)	DUTY (80 ppm)	DUTY (120 ppm)
1	0.5	4	8	12
3	0.5	8	14	20
1	1	4	8	12
5	1	10	20	27
2	2	8	14	20
5	2	10	20	28
10	2	55	Fail	Fail
3	3	9	15	23
10	3	60	Fail	Fail
5	5	10	20	28
10	5	60	Fail	Fail

CardioHELP HLS 7.0 ECLS SYSTEM:				
Sweep Gas (L)	Blood Flow (L)	DUTY (40 ppm)	DUTY (80 ppm)	DUTY (120 ppm)
1	0.5	5	10	14
3	0.5	9	18	25
1	1	5	10	14
5	1	16	29	42
2	2	8	15	21
5	2	15	36	54
10	2	70	100	Fail
3	3	10	18	25
10	3	70	Fail	Fail
5	5	13	23	33
10	5	70	Fail	Fail

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:**
      - Protocol
      - **Results/developments/achievements:**

b) **Objective 4.2:** Assess platelet activation and consumption during 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:**
   - Staff trained on instruments/techniques, awaiting start of animal studies.
   - **Results/developments/achievements:**
   - Studies will begin as soon as ACURO approval is achieved.

c) **Objective 4.**3: Assess systemic coagulation status during 24 hours ECLS in vivo with and without administration of NO gas into membrane oxygenators
   - **Major activities:**
   - Staff trained on instruments/techniques, awaiting start of animal studies.
   - **Results/developments/achievements:**
   - Studies will begin as soon as ACURO approval is achieved.

d) **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.
   - **Major activities:**
   - Staff trained on instruments/techniques, awaiting start of animal studies.
   - **Results/developments/achievements:**
   - Will be initiated with start of animal studies once ACURO approval achieved.

e) **Objective 4.**5: Assess circuit patency, pressures, and blood flow during 24 hours ECLS in healthy swine with and without NO gas adminsrtatino into membrane oxygenators.
   - **Major activities:**
   - Staff trained on instruments/techniques, awaiting start of animal studies.
   - **Results/developments/achievements:**
     Will be initiated with start of animal studies once ACURO approval achieved.

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:**
   - Staff trained on instruments/techniques, awaiting start of animal studies.
   - **Results/developments/achievements:**
   - Studies will begin as soon as ACURO approval is achieved.

-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.

*This Aim has not started as it requires a formal award modification to exercise and fund optional Phase 2, which is subject to availability of funds and satisfactory performance including meeting deliverables and government program approval.

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

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

At AREVA, post-baccalaureate students who are in the process of applying for medical school or other professional schools have had the opportunity to gain hands-on experience with ECLS systems. The students learn to adjust the ECLS system settings and also learn to operate the eNO generator through the ex vivo circulation test studies. The students will continue to be trained on ICU skills, anesthesia, vital signs recording and monitoring, blood gas collection and analysis, and coagulation tests during the animal studies. Dr. Roberts from Dr. Batchinsky's team had the opportunity to present the work performed on this project at several key meetings this year.

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 invited lectures by Dr. Roberts at the ASAIO annual meeting ("Coagulation management approaches for extracorporeal pulmonary support in the pre-hospital setting") and at the 35th Annual Meeting of the Japanese Association for the Surgery of Trauma ("Bio-inspired surfaces and regional anticoagulation strategies for extracorporeal life support: material assessment and development protocol for clinical translation"). The results of the NO generator testing were selected for presentation at the 2021 MHSRS meeting which was unfortunately cancelled.

Drs. Yu and Zapol have 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. Dr. Berra proposed that we collaborate on a project of study high dose NO as an antimicrobial in patients with respiratory infections. Dr. Stuart proposed that it will be interesting to study if we can use this portable eNO device to treat patients with high altitude pulmonary edema. A potential collaboration on this proposal is in progress.

What do you plan to do during the next reporting period to accomplish the goals?
In the next reporting period (months 13-15), MGH plans to accomplish the following objectives:
(1) Test the purity of electrically generated NO with ICP-MS and the efficiency of using scavenging-HEPA filtration system to remove all contaminations (e.g. NO derivatives and metal particles) in the electric NO.
(2) Transfer the portable eNO generator from MGH to Geneva/AREVA for blood circulation and in vivo testing.

In the next reporting period, AREVA plans to receive ACURO approval for 24-hour animal studies and to start animal studies as soon as the new generator from MGH is transferred to AREVA. Safety of the NO generator for use in the hyperbaric chambers will be assessed as well.

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.

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. Accumulating *in vitro and in vivo* evidence supports the use of high dose NO, a potent free radical and nitrosating agent, as an antimicrobial. 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*. 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*. Preliminary results from MGH's 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 short periods of 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 (as opposed to 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
Dr. Batchinsky's team submitted a proposal to modify the original study Specific Aim 2 to include a small pilot in vivo study rather than the originally planned ex vivo study – this proposal was

approved along with a revised SOW and budget. Since receiving approval the AREVA team drafted the new animal use protocol which was approved by IACUC and has now been sent to ACURO. We anticipate starting these experiments next quarter pending ACURO approval.

Actual or anticipated problems or delays and actions or plans to resolve them
At MGH, to measure the purity of electrically generated NO from the eNO device, we will need send all collected samples to a Mass Spectrometry Core Facility at UMass (outside MGH). We were informed that the turnaround time has been dramatically delayed due to COVID-19 causing short of onsite staff. As a long-time customer, we discussed this issue with the manager of the core facility and reached a reasonable and acceptable timeline for processing our samples. We do not expect any other problems or delays to the study timeline.

At AREVA, we are awaiting the completion of all NO generator screening tests at MGH and transfer of the generator from MGH to AREVA in order to start animal studies. In conversation with MGH, the generator will be transferred early in the next reporting period enabling us to begin animal studies ASAP in keeping with the SOW.

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.
  Nothing to report.

  Books or other non-periodical, one-time publications.
  Nothing to report.

  Other publications, conference papers and presentations.

  Invited Conference Lecture: Roberts TR, Batchinsky AI. Bio-inspired surfaces and regional anticoagulation strategies for extracorporeal life support: material assessment and

development protocol for clinical translation. *35th Annual Meeting of the Japanese Association for the Surgical Trauma (Virtual).* 28 May 2021.

Invited Conference Lecture: Roberts TR, Batchinsky AI. Coagulation management approaches for extracorporeal pulmonary support in the pre-hospital setting. *ASAIO 66th Annual Conference (Virtual);* 4 June 2021, Washington D.C., 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:	0.4
Contribution to Project:	Overseeing 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:	Jae Choi, PhD
Project Role:	Co-Investigator
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.3
Contribution to Project:	Carrying out the project protocol, preparation for pathological assessment and assays, and preparing manuscripts and reports.

Name:	Brendan Beely
Project Role:	Research Coordinator
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.3
Contribution to Project:	Performing routine laboratory procedures, assisting with study protocols, and preparing reports.

Name:	Dan Wendorff
Project Role:	Laboratory Manager
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.3
Contribution to Project:	Performing routine laboratory procedures, preparation of animal protocol and oversight of IACUC review, overseeing Lab. Techs.

Name:	George Harea
Project Role:	Research Associate
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.8
Contribution to Project:	Assisting with procurement requests, study design, and assisting with data collection and interpretation. Directing personnel in ex vivo studies. Design of ex vivo circuit.

Name:	Hailee Alaniz
Project Role:	Laboratory Technician
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.9
Contribution to Project:	Assisting with data collection and technical procedures. Assisting with preparations for animal testing. Assisting with ex vivo studies.

Name:	Clayton Smith
Project Role:	Laboratory Technician
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.9
Contribution to Project:	Assisting with data collection and technical procedures. Assisting with preparations for animal testing.

Name:	Ji Lee
Project Role:	Laboratory Technician
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.8
Contribution to Project:	Assisting with data collection and technical procedures. Assisting

	with preparations for animal testing. Assisting with ex vivo 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

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

QUAD CHARTS:
See attached.

9. APPENDICES:
N/A