CLINICAL PROTOCOL

Includes statistical analysis plan

Protocol Title:	Inhaled Nitric Oxide (iNO) after Out-of-Hospital Cardiac Arrest (OHCA)
IRB Protocol Number:	PRO16100408
NCT Number:	03079102
Version # and Date:	V11: July 25, 2019

NOVEMBER 30, 2021

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PROTOCOL SYNOPSIS

Protocol Title:	Inhaled Nitric Oxide (iNO) after Out-of-Hospital Cardiac Arrest (OHCA)	
Protocol Number:	PRO16100408	
NCT Number:	03079102	
Version # and Date:	V11: July 25, 2019	
Clinical Phase:	Phase II	
Investigational Drugs:	Nitric Oxide (Inhaled)	
Trial Site:	UPMC Presbyterian and Mercy Hospitals	
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Study Monitor:	Cameron Dezfulian, MD	
Research Facilities:	UPMC Presbyterian and Mercy Hospitals	
Clinical Laboratories:	UPMC	
Study Rationale:	iNO is FDA approved for the treatment of pulmonary hypertension in the setting of hypoxemic respiratory failure in neonates. It has been used off label extensively primarily for the treatment of hypoxemic respiratory failure and pulmonary hypertension with extensive safety data available for endpoints as far out as 2 years from use.[1-6] In these studies efficacy as measured by increase in oxygenation was noted with doses as little as 1 ppm[3,4] with few cases of methemoglobinemia or excess NO_2 especially at the doses under 40 ppm. A recent meta-analysis of trials of iNO to treat ischemia reperfusion injury in humans demonstrated efficacy in the majority of studies and an excellent human safety profile, but noted no existing human evaluation of iNO in the setting of acute brain injury including OHCA.[7] Effects of iNO after cardiac arrest has been studied in 4 preclinical animal studies.[8-11] These published studies demonstrated benefit using iNO 40 ppm started within 2h of	

	ROSC (Return of Spontaneous Circulation) which is additive to therapeutic hypothermia[8] as well as neuroprotection without temperature control.[9] These animal models include mouse and pig models of asystole and ventricular fibrillation (VF). In addition, iNO has been shown to protect in animal models of focal heart[12-15] and brain[16-19] injury. This is important since most of the mortality and morbidity after OHCA results from heart and brain injury.[20] Animal models of asphyxia[21] and other focal brain ischemia reperfusion models demonstrate NO deficiency early in reperfusion lasting at least 6h and benefits from iNO.[16,19] However in human brain injury from stroke[22,23] or trauma[24] the CSF (Cerebrospinal Fluid) levels of NO (Nitric Oxide) metabolites appear elevated by 24h with one study showing this effect is blocked using the iNOS (inhaled Nitric Oxide Synthase) inhibitor 1400W.[22] Based on these reports it would appear a state of NO deficiency is highly likely at ROSC and persistent for up to 24h after ROSC. Thus the likely therapeutic window is from ROSC until 24h after ROSC.
Study Objectives:	1. To evaluate the efficacy of inhaled nitric oxide dosed shortly after OHCA in reducing heart and brain injury after out-of-hospital cardiac arrest. 2. To examine a novel point of care method, laser speckle contrast imaging (LSCI) reactive hyperemia (RH), as a means of determining iNO responsiveness in the heterogeneous OHCA population to permit future screening if successful. 3. To determine the pharmacokinetics of systemic nitric oxide (NO) metabolites as a marker of endogenous and exogenous NO bioavailability in cardiac arrest survivors treated with iNO or placebo. 4. To determine which NO signaling pathways are activated by iNO and whether these are associated with clinical benefit.
Study Hypothesis:	Inhaled Nitric Oxide after OHCA will reduce heart and brain injury vs placebo
Study Aims:	1. Determine whether iNO (compared to placebo) provides clinical benefit when used within 4h of ROSC after OHCA. 2. Determine which mechanistic pathways are activated through use of iNO therapy and whether any of these are associated with outcome. 3. Determine whether iNO at 20 ppm provides a physiologic effect as determined by a significant reduction in tricuspid regurgitant velocity compared to placebo. 4. Determine whether RH measured by LSCI predicts subjects who will have a physiologic and/or clinical response to iNO.

	5. Determine whether iNO reduces oxidant stress and mitochondrial injury after OHCA compared to placebo. 6. Measure plasma nitrite and S-nitrosothiol levels, as a surrogate of total plasma bioavailable NO, in all subjects.
Study Design:	Phase II double blind (participants and investigator) placebo controlled randomized (1:1) clinical trial of iNO 20 ppm administered over 12 hrs beginning as soon as possible but within 4 hrs of ROSC after OHCA.
Planned Sample Size:	130 subjects
Duration of Treatment:	12h
Major Inclusion Criteria:	Intubated and comatose adult (>18 yo) Out-of-hospital cardiac arrest (OHCA) Cardiac arrest within an emergency department or outpatient medical center will be included. OHCA includes EMS witnessed cardiac arrest. ROSC within 40 min of CPR initiation FOUR Brainstem score ≥ 2 Patient must have pupil OR corneal reflex before enrollment

Major Exclusion Criteria:	- Traumatic etiology of OHCA
	- Prisoner
	- Known pregnancy
	  o B-HCG screening is NOT REQUIRED for enrollment in women of appropriate age
	- Hemodynamic instability defined as:
	  o > 1 recurrent arrest prior to enrollment
	  o Inability to maintain mean arterial blood pressure (MAP) > 65 using vasopressors and inotropes (ie actively up titrating medications or giving fluid bolus)
	- Cerebral edema defined as:
	  o Head CT grey-white ratio < 1.2
	  o Fixed and dilated pupils without another explanation
	- Malignant EEG upon presentation (not required as screening) defined as:
	  o Myoclonic status epilepticus
	  o Non-convulsive status epilepticus
	  o Generalized periodic epileptiform discharges
	- ROSC >3h from time of ED arrival (Treatment allocation must be within 4h so anything that will prevent this is reason for exclusion)
	- Alert and interactive patient with minimal evidence of neurologic injury

	- Plan to extubate within 12 hours
	- PCAS opinion that patient will die with >95% likelihood. This may be based on:
	  o Multiple medical comorbidities
	  o Late discovery of DNR or advanced directive
	  o Terminal diagnosis (other than OHCA; may have caused OHCA)
	  o Clinical judgement based on current exam and data
	- Known intracranial hemorrhage or acute cerebral infarction; Head CT is NOT REQUIRED prior to enrollment
	- Patient is known to be taking PDE5 inhibitors, sGC stimulator, or has a known diagnosis of Chronic thromboembolic pulmonary hypertension (CTEPH), pulmonary hypertension (PAH), or erectile dysfunction
	- Known enrollment in another acute interventional trial
	-

Study Endpoints:	**<u>Primary</u>** Composite of in-hospital death, unfavorable discharge location, NYHA class III/IV heart failure at the time of discharge* *In the setting of pre-existing heart failure or prior residence in an unfavorable location there must be at least a 1 class decrement (eg NYHA III -> IV) **<u>Secondary</u>** **Functional** -Survival to hospital discharge, 30 and 90 days after initial OHCA -Favorable cerebral performance category (CPC) defined as 1 or 2 at discharge, 30 and 90 days after initial OHCA -Favorable modified Rankin score (mRS) defined as 0-3 at discharge, 30 and 90 days after initial OHCA -Favorable discharge destination or location of residence (30 and 90 days after OHCA) defined as home or acute inpatient rehabilitation -CPC-E at hospital discharge, 30 and 90 days after initial OHCA -Barthel Index of Independence in Activities of Daily Living (ADL) at discharge, 30 and 90 days after OHCA **Neurologic** -Estimated likelihood of good neurologic outcome (0-100%) assessed by an experienced (>3y) member of the PCAS team blinded to treatment assignment on day 0-3 post-ROSC. -Development of malignant EEG during hospitalization

	-Time (in hours) to awakening from ROSC; defined as following commands (all values > 96h assigned 100) -FOUR Motor + Brainstem scores on days 1-3 (requires 30 min off sedation; exclude endpoint if not medically permitted to pause sedation or paralysis) -Presence or absence of upper and lower extremity somatosensory evoked potential N20 on comatose patients obtained any time after post-arrest day 1. - Absolute value 24h after ROSC and change in value (24h vs 0h) of neuron specific enolase (NSE) -EEG burst suppression ratio and change in suppression ratio -EEG presence of dominant continuous background and reactivity to photic or tactile stimulation. **Cardiovascular** -Heart rate and mean arterial pressure (preferably off the arterial line) at 0-13 (hourly), 24h of study drug -Persistent need for vasopressors in the first 24h after ROSC; defined as any use of norepinephrine, epinephrine, phenylephrine, dopamine or vasopressin for ≥3h after study drug is initiated -Persistent need of inotropes in the first 24h after ROSC; defined as any use of epinephrine, dobutamine, milrinone or isoproterenol for ≥3h after study drug is initiated. -ECHO measures (absolute values at end of therapy (12h) and change in 12h vs 0h): -tricuspid valve regurgitant velocity (TRV) -cardiac output based on aortic valve velocity (AV) -tricuspid annular plane systolic excursion (TAPSE) -left ventricular ejection fraction (EF). -Absolute value at end of therapy (12h), change in value (12h vs 0h) and peak 24h post-ROSC troponin I -Arterial blood gases at 0, 6, 12 and 24h after study drug initiation (when available) -Upper body central venous oxygen saturation at 0, 6, 12 and 24h after study drug initiation (when available) -Lactate at 0, 6, 12 and 24h after study drug initiation **Biochemical** -NO metabolites. Plasma nitrite, S-nitrosothiols and cyclic guanosine monophosphate (cGMP) levels obtained at 0, 3, 6, 9, 12h of study drug and 24h, 48h and 72h after study drug start. -RBC pellet for hemoglobin (Hb) studies (retained from above) - MetHb at 6h (safety endpoint) -Platelet mitochondria. Oxygen consumption rate, respiratory control ratio and superoxide generation (MitoSOX) at 0 and 12h

	of study drug when available. This data will be collected only when patients are enrolled from 6a-6p during weekdays (70/130 budgeted) to assure quality. -Plasma for ascorbate 0, 3, 12, 24h -Cardiolipin at 0, 3, 12, 24h -After obtaining informed consent (IC) banked blood for DNA (pharmacogenetics) -LSCI coupled to 1 and 5 min forearm vaso-occlusion at 0 h and 11 h. Endpoints will include ischemic trough, time to RH and RH peak, blood flow nadir 1 and 5 min after cuff release (respectively).

1. OBJECTIVE, SPECIFIC AIMS, BACKGROUND, AND SIGNIFICANCE

1.1 OBJECTIVE

Primary:

To evaluate the efficacy of inhaled nitric oxide (iNO) dosed shortly after out-of-hospital cardiac arrest (OHCA) in reducing heart and brain injury and death.

Secondary:

- To evaluate the efficacy of iNO in modulating surrogate markers of heart and brain.
- To evaluate the effects of iNO on platelet mitochondrial function.
- To examine a novel point of care method, laser speckle contrast imaging (LSCI) reactive hyperemia (RH), as a means of determining iNO responsiveness in the heterogeneous OHCA population to permit future screening if successful.
- To determine the pharmacokinetics of systemic nitric oxide (NO) metabolites as a marker of endogenous and exogenous NO bioavailability in cardiac arrest survivors treated with iNO or placebo.
- To determine which NO signaling pathways are activated by iNO and whether these are associated with clinical benefit.

1.2 SPECIFIC AIMS

Hypothesis: iNO after OHCA will reduce heart and brain injury thus reducing death when compared to placebo

Specific Aims:

Primary

Determine whether iNO compared to placebo provides clinical benefit when used within 4h of ROSC after OHCA.

Secondary

- Determine which mechanistic pathways are activated through use of iNO therapy and whether any of these are associated with outcome.
- Determine whether iNO compared to placebo provides a physiologic effect in the heart.
- Determine whether LSCI measures of endothelial function are associated with subjects who have a physiologic and/or clinical response to iNO (heterogeneity of treatment effect).
- Determine whether iNO reduces oxidant stress and mitochondrial injury after OHCA compared to placebo.
- Measure bioavailable NO and its downstream signals in the early post-arrest period in OHCA subjects treated or not treated with iNO.

1.3. BACKGROUND and RATIONALE

OHCA presently results in substantial mortality (~250,000 deaths annually in the US) due to brain and heart injury. iNO has shown promise in reducing brain and heart injury in numerous animal studies of focal ischemia-reperfusion injury (IR) and in animal studies of cardiac arrest. A recent meta-analysis of iNO in human studies targeting IR showed promise in the majority of trials. iNO has been used in dozens of human clinical trials with few adverse effects compared to placebo thus safety is not a major concern. iNO has never been examined as a therapy following human brain IR or OHCA thus its efficacy in this setting remains unclear. Since the clinical experience with iNO suggests good safety and the preclinical/clinical data suggests potential benefit, this represents a promising therapy for human OHCA brain and heart IR injury and warrants investigation.

1.4 SIGNIFICANCE

OHCA carries an extremely high mortality rate resulting from brain and heart injury as well as substantial morbidity in long term survivors. At present, there are no alternative therapies to mitigate this injury. Thus iNO could save lives and/or improve long term cardiac and/or neurologic function therefore addressing a significant public health concern lacking any current therapies.

2. RESEARCH DESIGN AND METHODS

2.1 CLASSIFICATION AND METHODOLOGICAL DESIGNS

The iNO OHCA study is a phase II double blind (participants and investigator) placebo controlled randomized (1:1) clinical trial of iNO 20 ppm administered over 12h (with 1 h weaning off) beginning as soon as possible but within 4 h of return of spontaneous circulation (ROSC) after OHCA. Planned enrollment is 130 subjects over 24 months at UPMC Presbyterian (PUH) and Mercy (MER) with randomization stratified in blocks of 8. Recruitment will be performed under exception from informed consent (EFIC) to facilitate early enrollment and treatment. The study will have a safety monitor to assess any concerns during its conduct and a pre-specified safety analysis at the mid-point (after 1 year or 60 patients whichever occurs first). Subjects or their surrogate (as subjects will be enrolled when comatose) will be notified as soon as possible by a member of the research team of enrollment and can elect whether to permit blood banking for future genetic studies.

Duration of subject participation is 90 ± 3 d of ROSC. Most data will be collected within four days of ROSC, which includes the 12h of study drug administration and 1h weaning of study drug. After the initial four days have passed follow up will be done at hospital discharge (DC; which includes death), day 30, and day 90 after OHCA to evaluate outcomes.

Subjects will not be withdrawn from or prevented from receiving any known effective therapy for the purposes of participating in this research.

2.2 DETAILED DESCRIPTION OF STUDY DESIGN

Study Visits and Procedures:

Timepoint	Task
Pre-Enrollment	Screening for inclusion and exclusion criteria
Before Administration of Study Drug, 0h	PCAS MD Baseline, Baseline lab data, ECHO and LSCI
During Study Drug Administration, 0h-13h	Vitals, Labs, ECHO, LSCI
Retrospective Baseline Data	Demographics, OHCA data, Clinical Lab data
Day 1 Post ROSC	VS, Labs, Clinical Lab ata, PCAS MD Daily data, Interventions, Medications, SOFA
Day 2 Post ROSC	VS, Labs, Clinical Lab ata, PCAS MD Daily data, Interventions, Medications, SOFA
Day 3 Post ROSC	VS, Labs, Clinical Lab ata, PCAS MD Daily data, Interventions, Medications, SOFA
Discharge	Survival, CPC, mRS, CPC-E, Barthel ADL, NYHA Class, Location
30d Follow Up	Survival, CPC, mRS, CPC-E, Barthel ADL, NYHA Class, Location
90d Follow Up	Survival, CPC, mRS, CPC-E, Barthel ADL, NYHA Class, Location

*****Please note that the 0h ECHO and LSCI may be collected up to one hour after the administration of the study drug to avoid issues with timing of enrollment.***

The majority of data, including all clinical lab data, that is collected for this study will be gathered during a single hospitalization. All labs will be collected within 30 minutes of their assigned time.

Follow-up data that will be obtained will not require a subsequent visit, but rather just a phone call.

Most of the baseline data elements can be effectively gathered retrospectively and will not be needed prior to starting the study drug. A small number of data elements will indeed need to be captured before study drug is administered but these can be done fairly rapidly to preclude delays in therapy onset. The same is true for data elements in days 1-3 post-arrest (0-24 h after ROSC is considered day 1)

Eligibility will be determined by a University of Pittsburgh Post-Cardiac Arrest Service (PCAS) physician, all of whom are routinely consulted in the care of this patient population and will serve as co-investigators on the study. Inclusion and exclusion criteria will be based on clinical characteristics (see attached Screening questionnaire). Due to the urgent need for intervention in this population, we will enroll subjects under an exception from informed consent (EFIC). Once eligibility is determined, patients will be randomized using block randomization to receive iNO or placebo. Randomization will be stratified in blocks of 8 by center. The randomization list will be created prior to the beginning of the study by the Respiratory Therapy (RT) directors (Edgar Delgado, PUH, and Robert Bizilla, MER) and confirmed by a representative of Mallinckrodt. These 3 individuals who will not be involved in any portion of the conduct or analysis of the study will retain these lists and update them as additional subjects are enrolled using new canisters of study drug. This list will set an order in which canisters of iNO study drug, coded by bar code, will be administered at each site. Treatment assignment will be made known to the study team after enrollment is confirmed by means of a sealed opaque envelope which contains the cylinder barcode for the subject as well as the subject ID. These envelopes will be with other research materials at each study site. iNO canisters will be stored in PUH Rm A-125 and Mercy (ED compressed gas storage). Prior to each enrollment the next canister to be used at a site will be loaded onto an iNOvent delivery device and the gas calibrated permitting rapid deployment to the next subject enrolled at that site. Check lists will be maintained as each site documenting calibration and each use on a study subject. The gas canister bar code will be recorded in our

database with the subject ID permitting subsequent unblinding of treatment assignment. Canisters will be used for 4 subjects prior to return to the company and replacement with a new (full) canister of study drug.

A clinical respiratory therapist (RT) will administer iNO at 20 ppm for 12h, with a 1h wean consisting of 10 min at 10 ppm, 10 min at 5 ppm, 10 min at 4 ppm, 10 min at 3 ppm, 10 min at 2 ppm, and 10 min at 1 ppm before subject is taken off of study drug. Study drug will generally be connected in the emergency room or ICU.

The following data elements will be collected during the course of the study at the times indicated. Data elements marked with an asterisk indicate variables or outcomes essential for conduct of the study and analysis of efficacy and/or safety. These data elements should be obtained in all subjects with no missing data expected. In addition to these data elements, we are collecting a large amount of secondary data intended for use in mechanistic studies and hypothesis generation. In many cases, these data elements WILL NOT be able to be collected in all subjects. Such missing data will be handled as designated within an a priori statistical analysis plan but the presence of missing data will in no way impede our ability to meet the objectives of this phase 2 RCT.

BASELINE DATA

- *Date of OHCA
- *Time of OHCA (If not available we will use Time of ROSC after subtracting estimated no flow and CPR time based on transfer records/physician report)
- Witnessed
- Bystander CPR
- EMS Call Date
- EMS Call Time
- EMS Arrival Date
- EMS Arrival Time
- No flow time
- CPR (low flow) time
- Presenting rhythm
- *Date and Time of ROSC (If not available we will use ED admission date/time from referring hospital record. If unavailable, stat medevac call date/time minus 30 mins. If unavailable, time of arrival minus 15 min)
- PCAS ID
- *Last name
- *First name
- *Date of Birth
- *Medical record number
- Marital status
- Race/Ethnicity
- *Male Sex
- *Height
- *Weight
- Post-resuscitation chest pain

- Charlson Comorbidity
- Age Adjusted for Charlson Comorbidity Index (CCI)
- *New York Heart Association (NYHA) class pre-OHCA (if no surrogate is present and no medical records are found on the subject this will be assumed to be NYHA class I)
- Barthel ADL pre-OHCA
- Education level
- Private insurance
- Medicare
- Medicaid
- Employed
- Active smoker
- Smoker >20 pack-years
- Prior stroke
- Prior Coronary artery bypass grafting (CABG)
- Noninsulin-dependent diabetes mellitus (NIDDM2) type 2
- Insulin-dependent diabetes mellitus (IDDM2) type 2
- Diabetes mellitus (DM1) type 1
- Prior myocardial infarction (MI)
- Congestive Heart Failure (CHF)
- Chronic Obstructive Pulmonary Disease (COPD)
- Prior cardiac arrest
- Dementia
- Hyperlipidemia
- Hypertension
- Prior coronary stent
- Obstructive sleep apnea
- Pulmonary hypertension
- *Location pre-OHCA
- *Best estimate of CPC pre-OHCA (if no surrogate is present and no medical records are found on the subject this will be assumed to be CPC 1)
- *Best estimate of mRS pre-OHCA ((if no surrogate is present and no medical records are found on the subject this will be assumed to be mRS 0)
- Rib fracture after ROSC
- Sternal fracture
- Witnessed aspiration
- Infection present at baseline
- Infection type
- Sputum color
- Vent mode
- Set Tidal Volume (Vt) or Inspiratory Pressure (Pinsp)
- Positive end expiratory pressure (PEEP)
- Fraction of inspired oxygen (FiO2) expressed as % oxygen
- Peak Pressure (PIP)
- Plateau Pressure (Pplat)
- Chest X-Ray reading possibly consistent with pneumonia
- Positive airway culture

Toxicology report
Cause of arrest
History of drug/alcohol use
Current drug/alcohol use

- ECHO RESEARCH ONLY To be performed by PCAS physician or research assistant trained by Marc Simon, MD, who is a cardiologist and Associate Professor of Medicine at the University of Pittsburgh.
  - Tricuspid valve regurgitant velocity
  - Aortic valve velocity (CO estimate)
  - Pulmonary valve velocity
  - Tricuspid annular plane systolic excursion (TAPSE)
  - LVEF
- *LSCI RESEARCH ONLY To be performed by a research assistant trained by the PI. A complete description is available in section CS10.1.
  - Baseline blood flow and RH after 1 min vasoocclusion [resolution 1 image/s; 10 image/s averaging]
  - Baseline blood flow and RH after 5 min vasoocclusion [begin 5 min after last test]*
- *Baseline labs (troponin, arterial blood gas, lactate, plasma nitrite level,)
- Additional plasma labs (neuron specific enolase, platelet mitochondria, ascorbate, cardiolipin, SNO, cGMP, plasma banking)
- Urine labs (renal failure and nitrated fatty acid measurements)
- Blood for genomics (retained unless informed consent declined)
- *Baseline vital signs VS (temperature, heart rate [HR], mean arterial blood pressure [MAP], arterial oxygen saturation [SpO2])

STUDY DRUG (0-12 h) DATA
(Refer to Chart below)

- *Every 3 hour vital signs VS (temperature, HR, MAP, SpO2)
- Hourly monitoring of VS, SAEs, vent settings
- Pulmonary Arterial Pressures (systolic, diastolic, mean) – often not available
- Central Venous Pressure – often not available
- Sv02 – often not available

- *Plasma labs at 6 and/or* 12 h (troponin, arterial blood gas, lactate, methemoglobin, plasma nitrite level)
- Additional plasma labs obtained variably at 3, 6, 9 and/or 12 h (neuron specific enolase, platelet mitochondria, ascorbate, cardiolipin, plasma banking)
- Urine labs at 0, 12 and 24 h (renal failure and nitrated fatty acid measurements)
- *LSCI (RESEARCH ONLY, as above)
  - 11-12 h RH after 1 min vasoocclusion [resolution 1 image/s; 10 image/s averaging]
  - 11-12 h RH after 5 min vasoocclusion [begin 5 min after last test]
- ECHO (RESEARCH ONLY, as above (11-12 h after study drug)
  - Tricuspid valve regurgitant velocity

- Aortic valve velocity (CO estimate)
- Pulmonary valve velocity
- Tricuspid annular plane systolic excursion (TAPSE)
- LVEF

Methemoglobin to be collected at 6 hours only

DAY 1-3 (0-72 h post-ROSC) DATA

During post-ROSC days 1-3, the following data elements will be collected by a physician coinvestigator/research assistant prospectively or by a research assistant retrospectively. Refer to chart for visual of when labs will be drawn.

- Plasma labs measured variably between 24 and 72 h (troponin, arterial blood gas, lactate, plasma nitrite level, neuron specific enolase, ascorbate, cardiolipin, plasma banking)
- Urine labs measured variably between 24 and 72 h (renal failure and nitrated fatty acid measurements)
- *Full Outline of UnResponsiveness (FOUR) Score (components below)
- Eyes
- Motor
- Brainstem
- Reflex
- *Glasgow Coma Score (GCS)
- Eyes
- Verbal
- Motor
- EEG interpretation
- Dominant background present
- Reactivity present
- Seizures present
- Seizure type
- Burst-suppression ratio
- Estimate of survival to hospital DC
- Median nerve SSEP
- Witnessed aspiration
- New suspected infection last 24h
- Infection type
- Sputum color
- Vent mode
- Set Vt
- Set PC
- PEEP
- FiO2 (Use % oxygen)
- Peak Pressure
- Plateau Pressure
- CXR last 24h read may be pneumonia
- Positive airway culture last 24h

- Vital Signs (VS) (highest and lowest for each)
- Time to follow commands (assigned once)
- SOFA (worst in prior 24h period; some components may be missing)
- Respiratory (when no PaO2 is available for PaO2:FiO2 ratio, use SpO2:FiO2 ratio)
- Nervous (based on GCS below)
- Cardiovascular
- Liver
- Coagulation
- Renal
- Interventions
- Coronary angiography
- PCI
- ECMO
- CPR
- CABG
- Mechanical ventilation hours
- NIPPV hours
- 24h FiO2 AUC
- Tracheostomy
- Medications (dichotomous for use not dose)
- Alteplase
- Anti-epileptic
- Midazolam infusion
- Intermittent Benzodiazapine
- Propofol infusion
- Intermittent Propofol
- Opiate infusion
- Intermittent opiate
- Neuromuscular blockade infusion
- Intermittent neuromuscular blockade
- Neurostimulants
- *Vasopressors
- *Inotropes
- Ketamine
- Steroids (hydrocortisone/methylprednisone)
- Cumulative vasopressor index (CVI; worst in prior 24h period)
- *BUN (highest value in prior 24 h)
- *Creatinine (highest value in prior 24 h)
- Hb
- WBC
- platelets
- PT
- INR
- PTT
- Bilirubin
- AST

- ALT
- BNP
- SvO2 (%)
- venous PaO2
- venous PaCO2

Research labs will be obtained by a study coordinator within 30 minutes of the assigned draw time. Labs will be run either within the UPMC Clinical Labs or the research labs of Drs. Dezfulian or Shiva as specified. We will also be collecting a small blood sample for an ancillary biomarker study for those subjects wishing to participate.

The final data to be collected will be the 90 d or discharge outcomes (whichever is later). Information that cannot be obtained by record review will be obtained either in person, or via a phone call by a member of the study team.

TABLE 1

	Time from study drug start (hours)							
Vital signs	0	3	6	9	12	24	48	72
Temperature	X	X	X	X	X	X	X	X
Heart Rate	X	X	X	X	X	X	X	X
Systolic Blood Pressure	X	X	X	X	X	X	X	X
Diastolic Blood Pressure	X	X	X	X	X	X	X	X
Mean Arterial Pressure	X	X	X	X	X	X	X	X
Arterial SBP	X	X	X	X	X	X	X	X
Arterial DBP	X	X	X	X	X	X	X	X
Arterial MAP	X	X	X	X	X	X	X	X
Pulmonary artery systolic pressure	X	X	X	X	X	X	X	X
Pulmonary artery diastolicpressure	X	X	X	X	X	X	X	X
Pulmonary artery mean pressure	X	X	X	X	X	X	X	X
Central venous pressure	X	X	X	X	X	X	X	X
Cardiac output	X	X	X	X	X	X	X	X
Vasopressors	X	X	X	X	X	X	X	X
Inotropes	X	X	X	X	X	X	X	X
Sedation	X	X	X	X	X	X	X	X
Neuromuscular blockade	X	X	X	X	X	X	X	X

	Time from study drug start (hours)							
Labs (highlight represents UPMC Labs)	0	3	6	9	12	24	48	72
Troponin	X		X		X			
Arterial blood gas (ABG)	X		X		X			
Lactate	X		X		X			
Methemoglobin			X					
Neuron specific enolase (NSE)	X					X		
Plasma NO metabolites	X	X	X	X	X	X	X	X
Platelet mitochondria	X				X			
Ascorbate	X	X			X	X		
Cardiolipin	X	X			X	X		
Inflammation (integrin α9β1 and CD11b)	X					X	X	X
Banked plasma	X	X	X	X	X	X	X	X
DNA for pharmacogenomics	X							
Whole blood pellet	X	X	X	X	X	X	X	X
Urine for renal failure markers and NO2-cLA	X				X	X		
*DNA will be collected with the first scheduled blood draw after obtaining informed consent								

Outcomes

The following will be recorded at DC (+/- 3 days), and for follow up at day 30 (+/- 3 days) and day 90 (+/- 3 days) after OHCA:

*Survival
Cause of death
date/time of death

*CPC
*mRS
CPC-E
Barthel ADL
*NYHA Class
*Location of residence

2.3 STUDY DRUG

2.3.1. Study Drug Preparation and Dispensing

iNO used in this study will be sourced and formulated by INO Therapeutics, part of Mallinckrodt Hospital Products IP Limited. Cross reference authorization has been granted to us by Mallinckrodt for information on the study drug, known as INOmax under New Drug Application (NDA) 20-845. Placebo gas wherein nitrogen (the carrier vehicle gas) is substituted for iNO will also come from Mallinckrodt.

Mallinckrodt will deliver gas cylinders to the Respiratory Therapy (RT) Department at UPMC PUH or MER. Each cylinder will have a bar code which corresponds to its contents (iNO or placebo). Randomization will be done in advance in blocks of 8 at each site by the RT directors using the website http://www.graphpad.com/quickcalcs/randomize1.cfm. The random order list generated will list the order in which subjects coming in to each site are assigned to cylinders of study drug. In each group of 8, 4 subjects will be assigned to one placebo cylinder and 4 subjects will be assigned to one iNO cylinder. RT will dispense study drug upon patient enrollment and the blinded research assistant (RA) will transport the cylinder/iNOvent, making note of its barcode to permit subsequent treatment assignment, to the patient's bedside for connection to the ventilator by RT. All patients will be on mechanical ventilation via an endotracheal tube (ETT). iNO is a commonly used drug within UPMC and existing protocols for its use will be followed in connecting the drug to the ventilator circuit.

2.3.2. Drug Administration

Research subjects will be comatose, so no specific instructions will be provided to them. RT will connect the study drug to the mechanical ventilator and the drug will be dosed via ETT for the entire 12 h period at 20 ppm followed by a 1 h wean. All patients will receive the same pattern of administration.

2.3.3. Dose Selection

A number of studies have reviewed the safety of iNO in adults primarily in the setting of acute respiratory distress syndrome (ARDS), A recent meta-analysis found improved oxygenation but also greater acute kidney injury (AKI) resulting from iNO use.[25] When this was examined by dose, AKI only resulted in those patients receiving higher cumulative doses of iNO (≥40 ppm for ≥48h).[26] Our study will limit iNO to 20 ppm for 12h hence AKI is not expected though we will track BUN and creatinine on all subjects. A separate meta-analysis examining effects of iNO in

acute hypoxemic respiratory failure in adults and children found no associated adverse events.[27] A study of iNO used in transport identified no adverse events associated with the drug.[28] Although iNO significantly increases methemoglobin (metHb) levels, the increase (0.3%) is clinically insignificant and not associated with toxicity [29] which is uncommon until levels of 20-30% (we will consider > 10% a significant and unexpected adverse event). The best predictor of significant metHb >4% (as < 0-3% is considered within normal range) is cumulative iNO dose of >2000 ppm * h.[30] In our study subjects will receive only 250 ppm * h. We will monitor methemoglobin as part of our study at 6h. Although iNO lowers pulmonary blood pressure it has not been linked to reduction in systemic arterial blood pressure. We will monitor blood pressure effects closely in our study given the inherent instability of cardiac arrest subjects.

Effects of iNO after cardiac arrest has been studied in 4 preclinical animal studies.[8-11] These published studies demonstrated benefit using iNO 40 ppm started within 2h of ROSC which is additive to therapeutic hypothermia[8] as well as neuroprotection without temperature control.[9] These animal models include mouse and pig models of asystole and ventricular fibrillation (VF). In addition iNO has been shown to protect in animal models of focal heart[12-15] and brain[16-19] injury. This is important since most of the mortality and morbidity after OHCA results from heart and brain injury.[20]

Animal models of asphyxia[21] and other focal brain ischemia reperfusion models demonstrate NO deficiency early in reperfusion lasting at least 6h and benefits from iNO.[16,19]

Information from prior clinical trials of iNO are available by cross reference in the Mallinckrodt NDA 20-845 (letter authorizing cross reference is included with application).

iNO is FDA approved for the treatment of pulmonary hypertension in the setting of hypoxemic respiratory failure in neonates. It has been used off label extensively; primarily for the treatment of hypoxemic respiratory failure and pulmonary hypertension. From these published studies, extensive safety data is available for a variety of endpoints with up to 2 year follow up.[1-6] In these studies efficacy as measured by increase in oxygenation was noted with doses as little as 1 ppm[3,4] with rare cases of methemoglobinemia or excess NO2 especially at the doses under 40 ppm. A recent meta-analysis of trials of iNO to treat ischemia reperfusion injury in humans demonstrated efficacy in the majority of studies and an excellent human safety profile, but noted no existing human evaluation of iNO in the setting of acute brain injury including OHCA.[7]

In human brain injury from stroke[22,23] or trauma[24] the CSF levels of NO metabolites appear elevated by 24h with one study showing this effect is blocked using the inducible NO synthase inhibitor 1400W.[22] Based on these reports it would appear a state of NO deficiency is highly likely at ROSC and persistent for up to 24h after ROSC. Thus the likely therapeutic window is from ROSC until 24h after ROSC.

Most studies that have demonstrated efficacy of iNO in animal models of CA or other IR injury have dosed the drug early (during CPR or at the time of ROSC). Only one study did a formal time course.[31] In this report iNO started 2h after CA improved neurologic function/injury and survival but 6h later did not. Thus the therapeutic window is between CPR onset and <6h after ROSC. One study showed efficacy at 1h after ROSC.[9] We selected a midpoint of 4h from ROSC as our latest time to initiate therapy but again stress that most data support earlier dosing either at the time of ROSC or during CPR. Thus our goal in designing a trial with recruitment under EFIC

is to enroll and dose patients as soon as possible after ROSC.

Our patients will generally achieve ROSC in the field (pre-hopsital). Most patients at PUH, our larger center, arrive as transports already 1-3h post-ROSC upon arrival. Thus at the time the subjects arrive in our center we will be under substantial time pressure to get them on study drug even to achieve the goal of dosing prior 4h of ROSC. Furthermore, **many of these patients are flown into our center or transported by ambulance with sirens activated, thus arriving without a family member from whom to obtain consent.**

2.3.4. Treatment Period

RT will administer iNO at 20 ppm for 12h, with a 1h wean consisting of 10 min at 10 ppm, 10 min at 5 ppm, 10 min at 4 ppm, 10 min at 3 ppm, 10 min at 2 ppm, and 10 min at 1 ppm before subject is taken off of study drug.

2.3.5. Breaking the Blind

The safety monitor can request unblinding in the case of any serious unanticipated adverse event (SUAE) based on clinical judgement and will unblind whenever there is a MetHb level > 10% associated with any adverse event. The safety monitor will review clinical data from the study consisting of VS, vasopressor and inotrope use, labs and outcomes, at least every 4 weeks. In the event of a SUAE, the safety monitor will review the record within 48 h breaking the blind to determine whether this is a drug associated SUAE.

The Data Safety Monitoring Board (DSMB) will meet prior to the commencement of the trial and every 6 months. All data will be reviewed at 12 months or 60 subjects for safety. The DSMB and safety monitor are authorized to unblind at any time during the conduct of the study if they have safety concerns. The investigators will remain blind up until the point that the DSMB decides to halt the trial or trial conclusion.

2.3.6. Medication Compliance

Since subjects are comatose and will be given study drug by endotracheal tube (ETT) over 12h compliance will be directly observed and verified by study team member in the research record. Thus, we anticipate 100% compliance unless study drug is stopped by a surrogate upon notification or for safety reasons both of which are unanticipated events.

2.3.7. Medication Storage and Accountability

Please refer to package insert of INOmax Nitric Oxide gas from INO Therapeutics/ Mallinckrodt for information on storage and accountability (cross reference authorized to NDA 20-845). Once delivered gas cylinders will be secured in a temperature controlled area within the respiratory therapy department. Gas cylinders (one blinded placebo and one blinded iNO) will be mounted on iNOvent delivery devices which will be stored within the emergency department to permit

rapid deployment. The contents of iNO after each use (in psi) will be documented and the cylinders will undergo high flow calibrations monthly and low flow calibrations weekly. Logs will be maintained on clipboards connected to the iNOvent.

2.3.8 Concomitant Medications

The following agents will not be permitted during study drug dosing: PDE5 inhibitors (sildenafil, vardenafil, tadalafil), sGC stimulators (riociguat), other nitrates (nitroglycerin, nitroprusside, isosorbide mono- and di-nitrate). PDE5 inhibitor and sGC stimulator use is cause for exclusion and these drug in general are seldom used within our population. We will likewise exclude subjects known to have the diagnosis of chronic thromboembolic pulmonary hypertension (CTEPH), pulmonary arterial hypertension (PAH) and erectile dysfunction which are the primary indications for PDE5 inhibitor and sGC stimulator use.

2.3.9 Rescue Medications

Hemodynamics will be stabilized using standard inotropic and vasopressor agents as needed in the ICU. There is no specific reversal agent for iNO. Methemoglobinemia can be reversed with treatment using methylene blue.

2.4 STUDY ENDPOINTS

Primary

Composite of in-hospital death, unfavorable discharge location, NYHA class III/IV heart failure at the time of discharge*

*In the setting of pre-existing heart failure or prior residence in an unfavorable location there must be at least a 1 class decrement (eg NYHA III -> IV)

Secondary

Functional

-Survival to hospital discharge, 30 and 90 days after initial OHCA

-Favorable cerebral performance category (CPC) defined as 1 or 2 at discharge, 30 and 90 days after initial OHCA

-Favorable modified Rankin score (mRS) defined as 0-3 at discharge, 30 and 90 days after initial OHCA

-Favorable discharge destination or location of residence (30 and 90 days after OHCA) defined as home or acute inpatient rehabilitation

-CPC-E at hospital discharge, 30 and 90 days after initial OHCA

-Barthel Index of Independence in Activities of Daily Living (ADL) at discharge, 30 and 90 days after OHCA

Neurologic

-Estimated likelihood of good neurologic outcome (0-100%) assessed by an experienced (>3y) member of the PCAS team blinded to treatment assignment on day 3-5 post-ROSC.

-Development of malignant EEG during hospitalization

-Time (in hours) to awakening from ROSC; defined as following commands (all values > 96h assigned 100)
-FOUR Motor + Brainstem scores on days 1-3 (requires 30 min off sedation; exclude endpoint if not medically permitted to pause sedation or paralysis)
-Presence or absence of upper and lower extremity somatosensory evoked potential N20 on comatose patients obtained any time after post-arrest day 1.
- Absolute value 24h after ROSC and change in value (24h vs 0h) of neuron specific enolase (NSE)
-EEG burst suppression ratio and change in suppression ratio
-EEG presence of dominant continuous background and reactivity to photic or tactile stimulation.

Cardiovascular
-Heart rate and mean arterial pressure (preferably off the arterial line) at 0-12 (hourly), 24h of study drug
-Persistent need for vasopressors in the first 24h after ROSC; defined as any use of norepinephrine, epinephrine, phenylephrine, dopamine or vasopressin for ≥3h after study drug is initiated
-Persistent need of inotropes in the first 24h after ROSC; defined as any use of epinephrine, dobutamine, milrinone or isoproterenol for ≥3h after study drug is initiated.
-ECHO measures (absolute values at end of therapy (12h) and change in 12h vs 0h):
  -tricuspid valve regurgitant velocity (TRV)
  -cardiac output based on aortic valve velocity (AV)
  -tricuspid annular plane systolic excursion (TAPSE)
  -left ventricular ejection fraction (EF).
-Absolute value at end of therapy (12h), change in value (12h vs 0h) and peak 24h post-ROSC troponin I
-Arterial blood gases at 0, 6, 12 and 24h after study drug initiation (when available)
-Upper body central venous oxygen saturation at 0, 6, 12 and 24h after study drug initiation (when available)
-Lactate at 0, 6, 12 and 24h after study drug initiation

Biochemical
-NO metabolites. Plasma nitrite, S-nitrosothiols and cyclic guanosine monophosphate (cGMP) levels obtained at 0, 3, 6, 9, 12h of study drug and 12h after cessation.
-RBC pellet for hemoglobin (Hb) studies (retained from above)
- MetHb at 6h (safety endpoint)
-Platelet mitochondria. Oxygen consumption rate, respiratory control ratio and superoxide generation (MitoSOX) at 0 and 12h of study drug when available. This data will be collected only when patients are enrolled from 6a-6p on weekdays (70/130 budgeted) to assure quality.
-Plasma for ascorbate 0, 3, 12, 24h
-Cardiolipin at 0, 3, 12, 24h
-After obtaining separate informed consent (IC) banked blood for DNA (pharmacogenetics)
-LSCI coupled to 1 and 5 min forearm vaso-occlusion at 0 h and 11 h. Endpoints will include ischemic trough, time to RH and RH peak, blood flow nadir 1 and 5 min after cuff release (respectively).

2.5 STATISTICAL ANALYSIS

2.5.1 Sample Size Determination

Sample size calculations will be based on the primary composite endpoint and ascertained via a two sample test of proportions. The control rate proportion was estimated to be approximately 80-85% and we have > 80% power to detect reduction in the composite endpoint of approximately 23% with 60 per arm and a type II error rate of 5%, and 65 per arm assuming minimal dropout in the range of 5%. Since our estimates of dropout are extremely conservative (our reported dropout in EFIC studies is <1%)[32] we are powered to detect changes in our primary composite endpoint as low as 21% if we actually observe little to no dropout. The secondary efficacy endpoint of iNO responsiveness is overpowered to detect a decrease in PA pressure in the region of 20-25 mm Hg with the planned enrollment.

2.5.2 Study Conduct Analysis

Continuous data will be summarized and compared using t-tests or a non-parametric equivalent should the normality assumption appear tenuous. Generalized linear mixed models, which account for non-independence of repeated assessments and endpoint structure, will be used to compare between iNO and placebo groups at multiple times. Analysis of the composite endpoint will be ascertained using an unadjusted two sample test of proportions with a type II error rate of 5%. In the event of imbalance of potential confounders after randomization an adjusted analysis will be performed using logistic regression with those unbalanced variables and treatment as the main effect of interest. Other secondary dichotomous outcomes will be summarized and compared by chi squared tests and appropriate exact methods should sample sizes be small. Time to event endpoints will be summarized by Kaplan Meir curves and compared using log rank tests. All tests will be two tailed with unadjusted $p<0.05$ considered significant and performed using SAS 9.4.

2.5.3 Efficacy Analysis

Our primary outcome is intended to prove efficacy. Improvement in any of the secondary clinical outcomes being assessed would be considered preliminary evidence of efficacy requiring a subsequent larger trial for proof. Since iNO's effects on pulmonary vasodilation are best known, we will use this as our primary physiologic surrogate measure of iNO efficacy and will be compared using unadjusted two-sample tests or adjusted regression based methods.

2.5.4 Safety Analysis

Section 6.4.5 outlines all potential SAE's and defines a SUAE as any SAE that occurs in the setting of MetHb > 10% which may thus be attributed to the study drug. All listed safety outcomes will be tabulated and summarized using basic descriptive statistics and tested using small sample exact methods.

2.5.5 Handling Missing Data

Since our primary endpoint is ascertained at hospital discharge we expect no missing data though all analyses will be performed assuming a missing at random mechanism. No data imputation will be performed though standard sensitivity analyses, using standard missing data techniques may be used.

2.5.6 Data Management

Subjects will be assigned a study ID upon entry and all data/samples stored using that ID. Linkage to patient identifiers will be maintained in a secure spreadsheet and will include name, DOB and MRN. De-identified clinical and lab data will all be stored in a web based database to be maintained by Dr. Dezfulian's research assistant who has prior experience from other studies. All data will be stored and managed with adherence to all relevant laws and HIPAA guidelines to ensure the safety and privacy of patient data.

3. HUMAN SUBJECTS

3.1 SUBJECT POPULATION

3.1.1 Inclusion of Women and Minorities

Women and Minorities will be included in the subject population

3.1.2 Inclusion of Children

Children will not be included in the subject population

3.1.3 Inclusion of Prisoners

Prisoners will not be included in the subject population

3.2 INCLUSION CRITERIA

Inclusion criteria do not change any subgroups.

Intubated and comatose adult (>18 yo)
Out-of-hospital cardiac arrest (OHCA)
Cardiac arrest within an emergency department or outpatient medical center will be included.
OHCA includes EMS witnessed cardiac arrest.
ROSC within 40 min of CPR initiation
FOUR Brainstem score ≥ 2
Patient must have pupil OR corneal reflex before enrollment

3.3 EXCLUSION CRITERIA

Exclusion criteria do not change any subgroups.

- Traumatic etiology of OHCA
- Prisoner
- Known pregnancy
    - B-HCG screening is NOT REQUIRED for enrollment in women of appropriate age
- Hemodynamic instability defined as:
    - > 1 recurrent arrest prior to enrollment
    - Inability to maintain mean arterial blood pressure (MAP) > 65 using vasopressors and inotropes (ie actively up titrating medications or giving fluid bolus)
- Cerebral edema defined as:

    - Head CT grey-white ratio < 1.2
    - Fixed and dilated pupils without another explanation
- Malignant EEG upon presentation (not required as screening) defined as:
    - Myoclonic status epilepticus
    - Non-convulsive status epilepticus
    - Generalized periodic epileptiform discharges
- ROSC >3h from time of ED arrival (Treatment allocation must be within 4h so anything that will prevent this is reason for exclusion)
- Alert and interactive patient with minimal evidence of neurologic injury
- Plan to extubate within 12 hours
- PCAS opinion that patient will die with >95% likelihood. This may be based on:
    - Multiple medical comorbidities
    - Late discovery of DNR or advanced directive
    - Terminal diagnosis (other than OHCA; may have caused OHCA)
    - Clinical judgement based on current exam and data
- Known intracranial hemorrhage or acute cerebral infarction; Head CT is NOT REQUIRED prior to enrollment
- Patient is known to be taking PDE5 inhibitors, sGC stimulator, or has a known diagnosis of Chronic thromboembolic pulmonary hypertension (CTEPH), pulmonary hypertension (PAH), or erectile dysfunction
- Known enrollment in another acute interventional trial

4. IRB APPROVAL AND FDA AMENDMENTS

The Investigator will obtain, from the University of Pittsburgh Institutional Review Board (IRB), prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and advertisements (i.e., directed at potential research subjects) for study recruitment.

The only circumstance in which a deviation from the current IRB-approved clinical protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research subject(s). In such circumstances, the Investigator will promptly notify the University of Pittsburgh IRB of the deviation. The Investigator should also notify the sponsor of this event.

The University of Pittsburgh IRB operates in compliance with FDA regulations at 21 CFR Parts 50 and 21 CFR 56, and in conformance with applicable International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice (CGP).

In the event that the University of Pittsburgh IRB requires, as a condition of approval, substantial changes to a clinical protocol submitted under an FDA-accepted IND application, or in the event of the Investigator's decision to modify the previously accepted clinical protocol:

- The Sponsor will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change to a Phase 2 protocol that significantly affects the safety of subjects, the scope of the investigation, or the scientific quality of the study. Examples of Phase 2 clinical protocol changes requiring the submission of a Protocol Amendment include:

    - Any increase in drug dosage or duration of exposure of individual subjects to the investigational drug beyond that described in the current protocol, or any significant increase in the number of subjects under study.

    - Any significant change in the design of the protocol (such as the addition or deletion of a control group).

    - The addition of a new test or procedure that is intended to improve monitoring for, or reduce the risk of, a side effect or adverse event; or the dropping of a test intended to monitor the safety of the investigational drug.

5. RECRUITMENT AND INFORMED CONSENT PROCEDURES

5.1 RECRUITMENT METHODS:

PCAS physicians are generally alerted to incoming resuscitated cardiac arrest patients either through a med call page (to accept the patient in transfer) or a page from emergency medical services stating the patient is en route to the emergency room (ER). PCAS physicians will review details of the patient's cardiac arrest to determine eligibility in consultation with a study coordinator. The ultimate designation of whether a patient is eligible will require a physical exam to determine neurologic function and hemodynamic stability upon ER presentation. If a patient is deemed eligible, they will be enrolled into the trial. We are requesting a waiver of informed consent to both review medical records to determine eligibility, and to enroll patients in the trial.

5.2 INFORMED CONSENT PROCEDURES

Since the study population is comatose (part of inclusion criteria), their surrogates are often difficult to locate or emotionally not able to provide consent and the therapy must be provided rapidly, recruitment will be done under EFIC. Subjects will be enrolled if they meet the eligibility criteria unless they have opted out of the study previously. Surrogates (the legally authorized healthcare representative [LAR]) will be informed that subject is enrolled in a trial at an appropriate time, based on the judgement of the study team, by either study coordinator or PCAS MD. If family chooses to withdraw consent, subject will be weaned off study drug. If the subject awakens, he/she will be informed of their participation in the study and that their records were reviewed. Banking of blood for genetic studies requires a separate informed consent. The genetic sample will not be drawn until consent for the procedure has been obtained. In the event that an LAR is identified but is unable/unwilling to come into the hospital for an in-person discussion, we are requesting the option to have a physician investigator discuss the study and go through the consent over the phone. We would then email a copy of the consent to the LAR and request that he/she respond that they are agreeable for the patient to participate in the genomic

portion of the trial, or indicate that they are not agreeable. We will have two people independent of the study, usually clinical nurses or physicians, witness the process. We would then draft a Note to File to keep in the subject's study folder outlining exactly what happened, along with a copy of the entire email discussion.

In the event that the LAR is unable/unwilling to come into the hospital and does not have access to email, we are requesting a waiver to document written informed consent for the notification of study enrollment only. The physician investigator will discuss the study and will obtain permission for continued participation. A Note to File will be placed in the subject's record documenting the discussion and the LAR's response.

6. POTENTIAL RISKS AND BENEFITS

6.1 POTENTIAL RISKS

6.1.1 Risk of Experimental Drug Intervention

The known adverse events due to iNO are reviewed in the package insert and in section 2.3.3 above. Extensive safety data is available from published studies of iNO in critically ill patients with up to 2 year follow up.[1-6] In these studies rare cases of MetHb or excess nitrogen dioxide [NO_2], an airway irritant, are reported. These cases occur at doses ≥40 ppm. The INOVent delivery system provides constant monitoring of NO_2 levels and we will monitor MetHb to avoid toxicity. iNO may cause rebound hypoxia after prolonged use but this generally requires over 24h of use. We plan a 1h wean off the study drug to minimize this risk. Other NO donors (not iNO) can reduce blood pressure but this has not been noted in human or animal studies of iNO at the doses we plan to use. Acute kidney injury (AKI) has been noted in some clinical trials of iNO but again only with higher doses than we propose to use. We will nonetheless monitor BUN and creatinine as markers of renal function. Patients receiving iNO may experience increasing capillary wedge pressure and worsening heart failure. We will monitor oxygen requirement (FiO_2) and saturation (SpO_2) hourly during study drug administration and the PI or a physician coI will be notified of an increase of >20% in the patients' oxygen delivery (mechanical ventilator FiO2) or a drop of > 50 mm Hg or 25% reduction (whichever is lesser) in the PaO2 if the patient is on FiO2 > 0.80. This data along with any change in chest X ray (generally obtained daily in these patients) and hemodynamics collected hourly and hourly fluid intake and output will also be reviewed for each patient by the safety monitor as described above (2.3.5).

6.1.2 Risk of Study Procedures

Blood will be obtained from pre-existing indwelling catheters required as part of patient care therefore there is no additional risk from phlebotomy. Likewise urine will be collected from existing foley catheters. The total whole blood being collected is 125 ml. This represents 2.5% of the average human blood volume and is not expected to result in any significant added stress to the subjects.

6.2 ALTERNATIVE TREATMENTS

No present FDA approved therapies to provide cardiac or neuroprotection after OHCA.

6.3 POTENTIAL BENEFITS

The risks listed here are minimal in comparison to the benefits which may be gained by reducing life ending brain and heart injury.

6.4 DATA SAFETY MONITORING

6.4.1 Data Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) will be created to review this study.

6.4.2 Data Safety Monitoring Plan

Data Monitoring Plan. The proposed study will use the FDA definition of adverse events (AE) and serious adverse events (SAE). Any SAE, which is unexpected and related to study intervention, will be reported immediately to the IRB and will be followed by an additional letter detailing the nature of the SAE. In the event that a participant either withdraws from the study or the investigators decide to discontinue a participant due to a SAE, the participant will be monitored by the co-PIs until (a) a resolution is reached (e.g., the problem has resolved or stabilized with no further change expected), (b) the SAE is determined to be clearly unrelated to the study intervention, or (c) the SAE results in death. Outcomes of SAEs will be regularly reported to the IRB and the sponsor. A summary of the SAEs that occurred during the previous year will be included in the annual progress report as well as in the annual IRB renewal.

Monitoring of safety and data quality in the proposed study will be the responsibility of all personnel on the project, with primary responsibility and supervision by the Investigator. The Institutional Review Board will approve the Notification / Statement of Informed Consent (IC) for the study and provide institutional oversight of data and safety issues. The study protocol will be approved prior to recruiting or obtaining consent from any participants. Moreover, the study will be reviewed at a minimum of annual basis (or more frequently as deemed necessary) by the IRB committee. Participants will be enrolled under EFIC and the LAR (and subject assuming awakening) will be provided notification of inclusion in the study and provided the option to withdraw IC. LAR/subject will separately be requested to provide IC for retention of blood for genomics. To ensure participant safety, once participants are enrolled in the study, study staff will immediately report all adverse and serious adverse events to one of the Investigators. The Investigator will, per standardized procedures, report them to the IRB for their review. These events should also be communicated to the sponsor of the IND. With regard to monitoring of data quality and protected health information, all required personnel proposed for this project will have the required human subjects and confidentiality training, which includes information about maintaining data integrity and security. Confidentiality will be guarded using established procedures such as storing data in locked cabinets within locked offices or locked data rooms, coding by study identification numbers rather than any personally identifying information to avoid revealing the identity of subjects, and aggregating data across participants. The key linking names and study identification numbers will be kept separately from the data sets with limited access by

study personnel (RAs and principal investigator only). Only study personnel will have access to the data sets on protected servers. Oversight of all aspects of data management will occur with the Investigator.

After initial approval and at periodic intervals (at least every 6 months; to be determined by the committee) during the course of the study, the DSMB responsibilities are to:

1. Review the research protocol, informed consent documents and plans for data and safety monitoring;
2. Evaluate the progress of the study, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, adverse events, unanticipated problems, performance of the trial sites, and other factors that can affect study outcome;
3. Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study;
4. Review clinical center performance, make recommendations and assist in the resolution of problems reported by the PI;
5. Protect the safety of the study participants;
6. Report on the safety and progress of the study;
7. Make recommendations to the PI, and if required, to the funding agency concerning continuation, termination or other modifications of the study based on the observed beneficial or adverse effects of the treatment under study;
8. Monitor the confidentiality of the study data and the results of monitoring;
9. Assist the PI by commenting on any problems with study conduct, enrollment, sample size and/or data collection.

The DSMB will include experts in critical care medicine, pharmacology, and biostatistics. Members will consist of persons independent of the investigators who have no financial, scientific, or other conflict of interest with the study. Written documentation attesting to absence of conflict of interest will be required.

The University of Pittsburgh Office of Clinical Research, Health Sciences will provide the logistical management and support of the DSMB. A safety officer (chairperson) will be identified at the first meeting. This person will be the contact person for serious adverse event reporting. Procedures for this will be discussed at the first meeting.

The first meeting will take place before initiation of the study to discuss the protocol, approve the commencement of the study, and to establish guidelines to monitor the study. The follow-up meeting frequency of the DSMB will be determined during the first meeting. An emergency meeting of the DSMB will be called at any time by the Chairperson should questions of patient safety arise.

The DSMB will meet prior to the beginning of enrollment and every 6 months until study completion (planned at 2 years). A planned interim analysis will be conducted at 1 year or after 60/130 subjects is enrolled, whichever occurs first.

6.4.3 Parameters to be Monitored

The following progress will be monitored throughout the course of the research to ensure the safety of subjects as well as the integrity and confidentiality of their data.

- An evaluation of the progress of the research study, including subject recruitment and retention, and an assessment of the timeliness and quality of the data.

- A review of collected data (including adverse events, unanticipated problems, and subject withdrawals) to determine whether there is a change to the anticipated benefit-to-risk assessment of study participation and whether the study should continue as originally designed, should be changed, or should be terminated.

- An assessment of external factors or relevant information (eg. pertinent scientific literature reports or therapeutic development, results of related studies) that may have an impact on the safety and study participants or the ethics of the research study.

- A review of study procedures designed to protect the privacy of the research subjects and the confidentiality of their research data.

The severity of adverse changes in physical signs or symptoms will be classified as follows:

- Grade 1 (Mild): asymptomatic or mild symptoms; clinical or diagnostic observation only; intervention not indicated.

- Grade 2 (Moderate): minimal, local or noninvasive intervention indicated; limiting age-appropriate ADL (Activities of Daily Living).

- Grade 3 (Severe): medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.

- Grade 4 (Life-threatening): consequences; urgent intervention indicated.

- Grade 5 (Death): event is a direct cause of death.

6.4.4 Frequency of Monitoring

The Investigator will review subject safety data as it is generated. The Investigator, sub-investigators, and the research staff will meet on a **monthly** interval to re-evaluate study goals, subject recruitment, data coding and retention, documentation and identification of adverse events, complaints and confidentiality of subjects. There will be an evaluation of the progress of the research study, including assessments of data quality, time lines, participant recruitment, accrual, and retention. The Investigator will also review the outcome and adverse event data to determine whether there is any change to the anticipated benefit-to-risk ratio of study participation and whether the study should continue as originally designed or

should it be re-evaluated and changed.

Independently the safety monitor will be review clinical data from the study consisting of subject VS, vasopressor and inotrope use and labs at baseline and during the first 24 h of enrollment (which includes study drug dosing) and final outcomes. This review will occur at least every 4 weeks. In the event of a SUAE, the safety monitor will review the record within 48 h, breaking the blind to determine whether this is a drug associated SUAE

6.4.5 Reportable Adverse Events

This is a high risk population in which numerous serious adverse events are expected. Indeed, all subjects will require mechanical ventilation, with >50% of subjects expected to require >50% FiO2 and/or vasopressors during the first 24 h based on our prior data[33,34] and expected mortality is 70-80%[35] as we are excluding non-comatose subjects. Thus numerous serious adverse events (SAE) are anticipated during the conduct of this study with a complete list provided below. In this context we define a SUAE as any SAE that occurs in the setting of MetHb > 10% which may thus be attributed to the study drug.

Expected SAE (with severity grade indicated)

- Non-central nervous system thrombosis, particularly in vessels accessed for endovascular devices, assessed as symptomatic venous or arterial thrombus documented radiographically or ultrasonographically. (Grade 3)
- Sepsis assessed as the presence of microbiologically proven, clinically proven, or suspected infection; presence of Systemic Inflammatory Response Syndrome (SIRS); and development of at least one organ dysfunction within the preceding 24 hours. Diagnosis of SIRS will require the fulfillment of at least two of the following criteria (Grade 4):
    - Hyperthermia (≥ 38°C) (which will only be evident after active temperature control);
    - Tachycardia (≥ 90 bpm);
    - Tachypnea (≥ 20 breaths/min) or an arterial pCO2 ≤ 4.3 kPa (32 mmHg) or mechanical ventilation;
    - White blood cell count ≥ 12,000/μL or ≤ 4,000/μL or left shift in differential white blood cell count ≥ 10%.
- Expected organ dysfunctions after OHCA (which may accompany SIRS in sepsis as defined above or separately) will be defined as:
    - Acute encephalopathy not explained by psychotropic medication (or attributable to the cardiac arrest) [Grade 3];
    - Thrombocytopenia ≤100.000/μL or drop in platelet count > 30% within 24 hours not explained by hemorrhage (Grade 3);
    - Arterial hypoxemia with an arterial pO2 < 8 kPa (60 mmHg) when breathing room air or an oxygenation index (paO2/FiO2 ≤ 200 mmHg) not explained by presence of a pulmonary or cardiac disease (Grade 4);
    - Arterial hypotension with a systolic blood pressure ≤ 90 mmHg or mean arterial blood pressure ≤65 mmHg for at least one hour despite adequate fluid loading not explained by other causes of shock (Grade 4);

    - Renal dysfunction with urine output ≤ 0.5 mL/kg/h for at least one hour despite adequate fluid loading or serum creatinine above 200 μmol/L (2.3 mg/dL) [Grade 3];
    - Metabolic acidosis with base deficit ≥ 5.0 mmol/L or serum lactate ≥ 1.5 fold above local reference range (2.4 mmol/L at UPMC) [Grade 3].
- Pneumonitis assessed as the presence of air bronchograms on any chest x-ray within one week of the index cardiac arrest. (Grade 3)
- Since many of these comatose OHCA subjects will receive therapeutic hypothermia, anticipated SAE include:
    - Electrolyte abnormalities: defined as the presence of any of the following from induction of hypothermia until 72 hours (all Grade 3):
        - Hypokalemia assessed as serum potassium concentration < 3.5 mmol/L.
        - Hyperglycemia assessed as serum glucose > 240 mg/dL.
        - Hypophosphatemia assessed as serum phosphate concentration < 0.8 mmol/L.
        - Hypocalcemia assessed as serum ionized calcium < 2.2 mmol/L.
    - Bleeding (overt bleeding with Hb drop > 3 g/dL or transfusion; cardiac tamponade; bleeding requiring surgical intervention for control or intravenous vasoactive agents; intracranial hemorrhage; intraocular bleed compromising vision; operation for vascular closure for the purpose of controlling bleeding; fatal bleeding) [Grade 3]
    - Thrombosis (Grade 3)
    - Infection (Grade 3)
    - Fluid shifts defined as net fluid intake/output exceeding 3 L positive or negative in a 24h period (Grade 3)
- Pulmonary Edema: We shall monitor the incidence of pulmonary edema in each treatment duration group and assess whether there is a significant difference between groups. It will be defined as the presence of congestive heart failure on a hospital-based chest x-ray (first emergency department or ICU chest x-ray). OHCA is more common in patients with cardiomegaly, and this radiographic sign alone will not be a criterion for diagnosis of acute heart failure. (Grade 3)
- Other common post-OHCA SAE (Grade 3 unless specified) include pneumonia, cerebral bleeding, stroke, seizures, bleeding requiring transfusion or surgical intervention, rearrest (Grade 5), rib fractures, sternal fractures, internal thoracic or abdominal injuries as well as any other major medical or surgical outcomes will be recorded as noted in the hospital discharge summary.

6.4.6 Adverse Events Reporting Timeline

All SUAE associated with the use of the study drug (ie in the setting of MetHb > 10% or discovered to be drug associated after unblinding by the safety monitor and considered potentially resulting from the study drug by the DSMB) will be reported to the IRB within 24 hours of discovery of the incident with subsequent follow-up submission of a detailed written report.

The FDA will be notified by telephone or facsimile transmission of a human SUAE no later than 7 calendar days after receiving the respective human adverse event information, followed by the

subsequent submission of a written IND Safety Report.

Serious and unexpected adverse events associated with the use of the study drug or procedures will be reported to the IRB with subsequent follow-up submission of a detailed written report in accordance with the respective policies and procedures of the IRB. Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the investigator-sponsor's receipt of the respective adverse event information.

A summary report of the findings will be prepared and submitted to the regulatory agencies.

6.4.7 Withdrawal of Subjects and Stopping Criteria:

Subjects withdrawn from the study due to concern for SAE will be followed for all clinical outcomes but no further lab or imaging data will be collected. These subjects will not be replaced within the study. Subjects will not be withdrawn after enrollment except if requested by the subject or surrogate after notification. In this event all data and specimens required for study outcomes obtained up to that time and clinical outcomes to DC will be retained.

Discontinuation of the Clinical Trial:
This study can be stopped only by the DSMB. In such a case we will complete collection of clinical outcomes in all enrolled subjects (up to 90 d after OHCA). Subjects will not be notified of early study termination.

6.5 RISKS MANAGEMENT PROCEDURES

6.5.1 Protection Against Risks

General Risks of Study Protocol and Procedures
All research interventions/activities will be conducted in private patient care areas. The collection of sensitive information about subjects is limited to the amount necessary to achieve the aims of the research, so that no unneeded sensitive information is being collected. Specifically we will only obtain 4 elements of protected health information (PHI) which could identify the subjects. These elements are the subject name (first, middle initial, last), date of birth and UPMC medical record number. These data elements will be stored in a password protected Microsoft Excel spreadsheet which links the PHI to the subject identifier (ID). The document will be stored on a UPMC cloud server drive accessible only to the principal investigator (PI) and data manager. This folder lies behind the UPMC firewall and hence has all security provisions attributable to the electronic medical record associated with it.

Those data elements which will be obtained by direct observation or physical examination of subjects will initially be recorded on a Case Report Form (CRF) which will be linked to the subject using the subject ID only. The Investigator will review, approve and sign/date each completed CRF; the Investigator's signature serving as attestation of the Investigator's responsibility for ensuring that all clinical and laboratory data entered on the CRF are complete, accurate and authentic. These data elements will subsequently be transferred by a research assistant to the

electronic database described below. The completed CRF will be stored in a locked filing cabinet within the locked office of the PI.

All staff will sign confidentiality statements. Access to the database will be limited to the data manager and staff under the supervision of the Investigator.

Specimens will be only be referenced by subject ID as described above. These specimens will be stored safely in the custody of the Investigator responsible for the individual assays. The Investigators will limit future access to any remaining sample to only those investigators with prior IRB approval for their studies.

All staff involved in this study are properly credentialed and instructed in the areas of testing, confidentiality, and safety.

The Investigator will retain the data for the entire period of this study and will retain the specified records and reports for up to two years after the marketing application is approved for the investigational drug; or, if a marketing application is not submitted or approved for the investigational drug, until two years after investigations under the IND have been discontinued and the FDA so notified. The Investigator may continue to use and disclose subjects' de-identified information for the purpose of this study for a minimum of seven years after final reporting or publication of the study. If the subject and/or legal representative decide to withdraw or be withdrawn from study participation, they may request that the study data and samples be destroyed. Subject names or other directly identifiable information will not appear on any reports, publications, or other disclosures of clinical study outcomes.

6.5.2 Protection Against Potential Risks of Experimental Intervention

The study drug (iNO) and administration system (INOVent) are already in clinical use within the UPMC system in the ICUs where subjects will receive care. Existing protocols for administration will be followed by trained RTs. The investigators are all qualified to perform the elements of data acquisition whether by exam, observation or phlebotomy as described below (8.1). All members of the study team will have received required training on protection of human research participants (CITI Good Clinical Practice Module) and all have prior clinical trial experience. During study drug administration the patient's VS, need for vasopressor/inotropic medications, ventilator settings, fluid balance and labs will be reviewed by a research assistant present throughout administration who will report any adverse events to a physician member of the PCAS team (on call 24/7). The PCAS co-investigators are empowered to stop study drug based on their discretion as is the safety monitor who can be consulted if needed. The safety monitor will review data on VS, need for vasopressor/inotropic medications, ventilator settings, fluid balance and labs at least every 4 weeks and data reviewed at least every 6 months by the DSMB.

7. COSTS AND PAYMENTS

7.1 COSTS

No additional cost to patients or insurers for study drug or labs and data collected as part of this study.

7.2 PAYMENTS

No compensation for study participation.

8. QUALIFICATIONS AND SOURCE OF SUPPORT

8.1 QUALIFICATIONS OF THE INVESTIGATORS

Cameron Dezfulian, MD, is an Assistant Professor of Critical Care Medicine and Clinical and Translational Sciences. He is an experienced researcher in cardiac arrest heart and brain injury, post-resuscitation care, nitric oxide and free radical biology and mitochondrial function. He is a Post Cardiac Arrest Service Physician at UPMC Presbyterian-Shadyside and routinely cares for the patient population being studied in this project. He also has the scientific background and lab resources as part of the Safar Center for Resuscitation Research to perform the studies planned.

Jon Rittenberger, MD, MS is an Assistant Professor of Emergency Medicine and an experienced researcher in acute neurological emergencies. He is a Post Cardiac Arrest Service Physician at UPMC Presbyterian-Shadyside and routinely cares for cardiac arrest patients as part of his clinical duties.

Clifton W. Callaway, MD, PhD - Professor and Vice-Chair in Emergency Medicine, Consultant on Post-cardiac arrest care. Dr. Callaway has career focus on cardiac resuscitation, acute brain injury, and also cares for cardiac arrest patients as part of his normal clinical duties.

Dr. Elmer, Dr. Guyette, Dr. Frisch, Dr. Weissman, Dr. Molyneaux, Dr. Shutter, Dr. Sawyer, Dr. Emlet and Dr. Doshi are all attending physicians in Emergency/Criticial Care Medicine. They routinely care for cardiac arrest patients as part of their clinical duties, and have taken part in several research projects within the department.

Dr. Okubo is an emergency medicine physician who is presently performing a clinical and research fellowship in the area of cardiac arrest resuscitation and thus also has expertise with this patient population.

Ms. Flickinger, MS, is an exercise physiologist and research specialist in the Applied Physiology Lab. Ms. Repine is also a research specialist in the Applied Physiology Lab. Both specialists have at least 3 years of experience in the human physiology lab and are trained in IV placement and obtaining blood draws. They both have experience in physiologic monitoring and thermoregulation.

Sara DiFiore is the study coordinator for the Department of Emergency Medicine. She has several years experience in the regulatory field and has participated in many of the department's

clinical trials.

Nick Krehel is a research assistant employed by Dr. Dezfulian. He previously worked in the laboratory of Dr. Dezfulian where he participated in several translational projects. He is adept at processing blood samples, a number of basic lab techniques (such as measure of NO metabolites being used in this study) and has been trained to conduct clinical research by Dr. Dezfulian. He will gather much of the data used in this study.

Dylan Burbee is a new research assistant employed by Dr. Dezfulian. He previously worked in a University of Pittsburgh laboratory where some of his duties included processing blood samples and a number of basic lab techniques. He was trained to perform ECHO and LSCI as well as how to collect clinical data for this study and has become the administrator of the REDCap database.

8.2 SOURCES OF SUPPORT

Investigator initiated research award from Mallinckrodt Pharmaceuticals.

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