BRIGHAM AND WOMEN'S HOSPITAL | Harvard Medical School

Rebecca M. Baron, M.D.
Associate Professor of Medicine.
Division of Pulmonary & Critical Care Medicine
75 Francis Street
Boston, MA 02115
(617) 525-6642
rbaron@bwh.harvard.edu

January 5, 2026

NCT04870125
A Phase 1b Study of Inhaled CO for the Treatment of Sepsis-Induced ARDS
Submission of Protocol/Statistical Analysis Plan (dated July 21, 2023) to allow us to submit study results

We are ready to submit our final results (and we need to do so as soon as possible), and we understand that you first need to approve and release our Study Protocol (dated/approved July 21, 2023) that includes our SAP. We attach these documents and hope that these can be approved and released as soon as possible to enable us to submit our final results by the required deadline.

Sincerely yours,

Rebecca M. Baron, M.D.

A Phase Ib Trial of Inhaled Carbon Monoxide for the Treatment of Pneumonia and Sepsis-Induced Acute Respiratory Distress Syndrome (ARDS)

National Clinical Trial (NCT) Identified Number: NCT04870125
Principal Investigator: Rebecca Baron, MD
IND Sponsor: Mark Perrella, MD

Funded by: National Heart, Lung, and Blood Institute (NHLBI)
Version Number: v.3.0
July 21, 2023

Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
Cover page	The protocol title, version, and version date have been updated	Administrative changes
Multiple Sections	Expanded inclusion criteria to include 3 strata: 1) ARDS or 2) pneumonia (unilateral or bilateral pulmonary infiltrates) without ARDS or sepsis, or 3) pneumonia (unilateral or bilateral pulmonary infiltrates) with sepsis, without ARDS.	Outcomes of patients with lung infections related to pneumonia have been reported to be similar with unilateral as for bilateral infiltrates, and this permits us to expand inclusion criteria to improve subject enrollment as well as conform to our preclinical primate FDA-enabling studies.
1.1 Synopsis	Pneumonia was added to the title, study description, objectives, and study population.	Change made to update the study population.
1.4 Definitions	The following language was added: **Pneumonia**: **Unilateral or bilateral pulmonary infiltrates in a patient with suspected respiratory infection.**	This change was made to clarify the definition of pneumonia.
2.1 Background	Added: **In April 2023, we elected to expand inclusion criteria to include intubated patients with pneumonia (unilateral or bilateral pulmonary infiltrates consistent with lung infection), given reported similar outcomes in critically ill patients with unilateral and bilateral infiltrates (LUNG SAFE study).**	This change was made to support the rationale for adding intubated patients with pneumonia to the study population.
2.2.1 Introduction	Added: **Pneumonia represents a lung infection with unilateral or bilateral infiltrates, and intubated patients with pneumonia not formally meeting ARDS criteria have been reported to have similar**	This change was made to support the rationale for adding intubated patients with pneumonia to the study population.

	outcomes with unilateral or bilateral infiltrates. Pneumonia in intubated patients represents an important spectrum of illness in ARDS, and in fact the most recent NIH lung injury consortium to improve phenotyping in critical illness will enroll subjects with pneumonia, sepsis and ARDS. Furthermore, our preliminary data demonstrating efficacy and safety of inhaled CO in pneumonia baboons is presented below.	
3.0 Objectives, 4.1 Overall Study Design, 4.2 Scientific Rationale	Objectives were updated to include pneumonia and/or sepsis in the study population.	Change made to update the study population.
5.1 Inclusion Criteria	The following changes were made to this section. All patients (age 18 and older) will be eligible for inclusion if they meet all of the following consensus criteria for sepsis and ARDS3,**or if they meet the criteria for pneumonia.** The following inclusion criteria was added: • **Pneumonia (without ARDS or sepsis) will be defined as a unilateral or bilateral lung infiltrate on chest X-ray or chest CT (not fully explained by effusions, lobar/lung collapse or nodules) in the setting of receiving mechanical ventilation, a new suspected respiratory infection, an increase in SOFA score less than 2 at the time of randomization (baseline). Pneumonia with bilateral opacities, PaO2/FiO2 ratio ≤ 300, or an increase in SOFA score greater than or equal to 2 over baseline will continue to be considered ARDS and sepsis.** • **Pneumonia (with sepsis, without ARDS) will be defined as a unilateral or bilateral lung infiltrate on chest X-ray or chest CT (not fully explained by effusions, lobar/lung collapse or nodules) in the setting of receiving mechanical ventilation and a new suspected respiratory infection with an increase in SOFA score of ≥ 2 over baseline at the time of randomization. Pneumonia with bilateral opacities, PaO_2/FiO_2 ratio ≤ 300, or an increase in SOFA score greater than or equal to 2 over baseline will continue to be considered ARDS and sepsis.**	Inclusion criteria revised to include intubated patients with pneumonia.

5.4 Strategies for Recruitment and Retention	The following language was added to this section: **We have expanded inclusion criteria to include subjects with unilateral or bilateral infiltrates intubated for suspected lung infection (pneumonia), given similar outcomes from unilateral or bilateral lung infiltrates in critically ill patients, consistency with our preclinical baboon model of ALI due to pneumococcal pneumonia that had improved outcomes with inhaled CO, and the need to expand enrollment for this study.**	This phase 1b protocol will also include subjects with unilateral or bilateral infiltrates intubated for suspected lung infection (pneumonia). This was added given similar outcomes from unilateral or bilateral lung infiltrates in critically ill patients and for consistency with our preclinical baboon model of ALI due to pneumococcal pneumonia that had improved outcomes with inhaled CO. This may also help with enrollment in this study.
9.1 Statistical Hypothesis	The following changes were made to this section. Hypothesis: We hypothesize that low dose iCO will be safe and well-tolerated and that the CFK equation-based iCO personalized dosing algorithm will be accurate in achieving a target COHb level of 6-8% in patients with **pneumonia (without ARDS and sepsis criteria, and with sepsis but without ARDS criteria) and** sepsis-induced ARDS **at study entry.** We further hypothesize that precision-based iCO therapy will reduce the severity of **pneumonia and** ARDS and organ failure by suppressing mitochondrial dysfunction, inhibiting inflammasome activation and necroptosis, and accelerating resolution of inflammation.	This change was made to incorporate the additional strata into the statistical plan.
9.4.2 Analysis of the Primary Efficacy Endpoint(s), 9.4.5 Safety Analysis	The following language was added: **ANOVA will be repeated to assess the effects of treatment, time (over 3 days), entry criteria (ARDS/Sepsis vs. Non-ARDS/Sepsis Pneumonia vs. Non-ARDS/non-Sepsis Pneumonia), and two-way/three-way interactions of time, treatment and entry criteria.**	This change was made to incorporate the additional strata into the statistical plan.
9.4.3 Analysis of the Secondary Endpoint(s)	This section was updated to add ARDS/Sepsis, Non-ARDS/Sepsis Pneumonia, and Non-ARDS/non-Sepsis Pneumonia to the analysis description for the secondary endpoints.	This change was made to incorporate the additional strata into the statistical plan.

CONTENTS

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56 and 21 CFR Part 312)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

I confirm that I have read and understand the protocol referenced above. I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission from the Protocol Chairs and the IRB.

Institution Name: _____

Site Principal Investigator Name: _____

Site Principal Investigator Signature: _____

Date: _____

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: A Phase Ib Trial of Inhaled Carbon Monoxide for the Treatment of Pneumonia and Sepsis-induced Acute Respiratory Distress Syndrome (ARDS)

Study Description: Multi-center, prospective, randomized, partially double-blind, placebo-controlled Phase Ib safety clinical trial of inhaled CO (iCO) for the treatment of pneumonia and sepsis-induced ARDS.

Hypothesis: We hypothesize that low dose iCO will be safe and well-tolerated and that the Coburn-Forster-Kane (CFK) equation-based iCO personalized dosing algorithm will be accurate in achieving a target carboxyhemoglobin (COHb) level of 6-8% in intubated patients with pneumonia and sepsis-induced ARDS.

Objectives: **Primary Objective:** To evaluate the safety and accuracy of a CFK equation-based personalized iCO dosing algorithm in intubated patients with pneumonia and sepsis-induced ARDS.

Secondary Objectives: To examine the effects of low dose iCO therapy on biologic readouts in intubated patients with pneumonia and sepsis-induced ARDS.

Endpoints: **Primary Endpoints:** The primary endpoints are:

1. **Safety of iCO**, defined by the incidence of pre-specified administration-related adverse events (AEs) and serious adverse events (SAEs). Administration-related SAEs are: 1) myocardial infarction; 2) stroke; 3) new onset arrhythmia requiring DC cardioversion; 4) worsening hypoxemia[1,2]; 5) COHb ≥ 10%; and 6) increase in lactate by ≥ 2 mmol/L.
2. **Accuracy** of the CFK equation-based personalized iCO dosing algorithm to achieve a COHb level of 6-8%.

Secondary Endpoints:

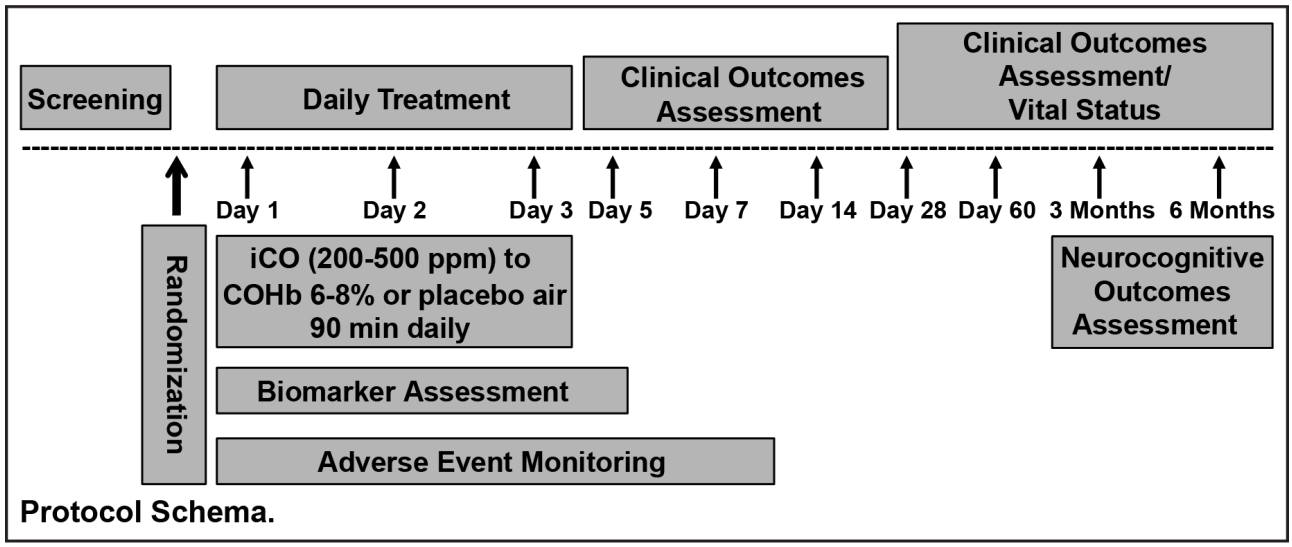
1. Lung Injury Score (LIS) on days 1-5 and day 7
2. PaO2/FiO2 on days 1-5 and day 7
3. Oxygenation index (OI) on days 1-5 and day 7
4. Dead space fraction (Vd/Vt) on days 1-3 and day 7
5. Sequential Organ Failure Assessment (SOFA) scores on days 1-5, 7, 14, and 28
6. Ventilator-free days at day 28
7. ICU-free days at day 28
8. Hospital-free days to day 60
9. Hospital mortality to days 28 and 60
10. Neurocognitive function at 3 and 6 months

Exploratory Endpoints:

1. Blood and urine biomarkers of mitochondrial dysfunction (mtDNA), inflammasome activation (IL-18) and necroptosis (RIPK3)

	2. Lipid mediators (LM) and specialized pro-resolving mediators (SPMs)
Study Population:	This study will enroll 36 adult subjects. All intubated patients ≥ 18 years old who meet criteria for pneumonia and/or sepsis and ARDS according to consensus criteria[3,4] will be potentially eligible for inclusion. Subjects will be recruited from the medical, surgical, and cardiac intensive care units (ICUs) at each center.
Phase:	Phase Ib
Description of Sites/Facilities Enrolling Participants:	The trial will be conducted at 6 tertiary care medical centers including Weill Cornell Medicine/New York-Presbyterian Hospital, Brigham and Women's Hospital (BWH), Massachusetts General Hospital (MGH), Duke (Duke University Hospital/ Durham Regional Hospital), Washington University, and New York-Presbyterian Brooklyn Methodist Hospital.
Description of Study Intervention:	Participants will receive algorithm-specified iCO dose (not to exceed 500 ppm) or medical grade air, to achieve COHb of 6-8% over 90 minutes for three consecutive days.
Study Duration:	Enrollment will take place over 27 months followed by 9 months for data analysis and dissemination of study results.
Participant Duration:	6 months

1.2 SCHEMA



Protocol Schema.

1.3 SCHEDULE OF ACTIVITIES (SOA)

Measurement/Event	**Baseline**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 7**	**Day 14**	**Day 28**	**Day 60**	**3M**	**6M**	**Post-discharge Call[K]**
Demographic/Admission Data/Medical Hx	X												
Etiology of ARDS	X												
Physical Exam	X												
Height/Body weight	X												
Time on ventilator prior to enrollment	X												
Pregnancy test	X												
Vital Signs (HR, SBP, DBP, MAP, T) [B]	X	X	X	X	X	X	X						
Central venous pressure [B]	A	A	A	A	A	A							
Electrocardiogram (EKG)	X	X	X	X	A	A	X						
Ventilator parameters [B, C]	X	X	X	X	X	X	X	X					
Chest X-ray (CXR) review [D]	X	A[D]	A[D]	A[D]	A[D]	A[D]	X						
Lung Injury Score (LIS)	X	X	X	X	X	X	X						
APACHE II Score	X												
SOFA Score [E]	X	X	X	X	X	X	X	X	X[F]				
Glasgow Coma Score (GCS)	X	X	X	X	X	X	X	X	X[F]				
Richmond Agitation Sedation Scale (RASS)	X	X	X	X	X	X	X						
Arterial Blood Gas (ABG)	X												
ABG; Venous Blood Gas if ABG not available [C]		X	X	X	A	A	A	A					
Sp02	X	X	X	X	X	X	X	A					
$Scv0_2$	A	A	A	A	A	A							
Complete blood count (CBC) [C]	X	X	X	X	A	A	A	A	A				
Basic metabolic panel [C]	X	A	A	A	A	A	A	A	A				
PT/PTT/INR [C]	A	A	A	A	A	A	A						
Serum CK, AST, ALT, Albumin, Total Protein [C]	A	A	A	A	A	A	A						
Total Bilirubin [C]	X	X	X	X	X	X	X	X	X				
Lactate [C]	X	X	X	X	A	A	A						
CO Administration Parameters / Treatment		X[G]	X[G]	X[G]									
Pulmonary Dead Space (Vd/Vt) Measurement		X	X	X			X						
COHb and SpCO		X[G]	X[G]	X[G]									
Vasopressors and inotropes [B]	X	X	X	X	X	X	X	X	X				
Fluid intake and output [B]	X	X	X	X	X	X							
Renal replacement therapy status	X	X	X	X	X	X	X	X	X				
Concomitant medications	X	X	X	X	X	X	X						
Adverse event monitoring [H]		X	X	X	X	X	X [H]						

Measurement/Event	Baseline	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 14	Day 28	Day 60	3M	6M	Post-discharge Call[K]
Plasma isolation		X	X	X		X							
RNA banking		X	X	X		X							
DNA banking – Cell pellet isolation		X	X	X		X							
Primary cell (PBMC) isolation		X				X							
Urine sample collection		X	X	X		X							
Discarded BAL and plasma [I]	A	A	A	A	A	A	A						
Microbiological results [J]	A	A	A	A	A	A	A	A	A				
Vital Status									X	X	X	X	X
Discharge Status									X	X			X
Ventilator Status									X	X			X
Vasopressor Status									X	X			X
Neurocognitive Outcomes-MoCA-BLIND, Hayling											X	X	

X - Required
A - When available
B - Data gathered at times indicated or until patient achieves 48 hours of unassisted breathing, whichever is sooner. Data will be obtained pre and post-treatment on study drug administration days.
C - Measure during reference period (03:00-10:00), obtain at time closest to study drug administration if applicable
D - CXRs done for clinical purposes will be reviewed on days 1-5. If the subject is extubated during days 1-5, a chest X-ray will be ordered by the study team the day following extubation, if not ordered by the clinical team.
E - Record clinically available creatinine, platelets, bilirubin, SBP and vasopressor use
F - Gathered on day 28 or on discharge date
G - Daily on days of study drug administration
H - AEs will be captured until study day 7
I - Obtained from discarded biobank (site specific) if available
J - Microbiological results recorded as they become available
K– If the subject is discharged home or to a long-term acute care facility before Day 28 or 60, he or she will be contacted by phone.

1.4 DEFINITIONS

Acute Kidney Injury: Acute kidney injury network Stage 3 disease, defined as a threefold increase in creatinine from baseline or the need for dialysis

Completing 48 hours of UAB (from weaning form): Defined as the date (calendar day) that the subject reaches exactly 48 hours of UAB. Example: if subject meets UAB at 1900 on 6/1/06 and does not return to AB, then the date of completing 48 hours of UAB would be 6/3/06.

Date of first UAB (from Study Termination form): Defined as the first day that the subject is on UAB from midnight to midnight. Example: if subject meets UAB at 1900 on 6/1/06, then the date of first UAB would be 6/2/06, as long as subject does not return to AB on 6/2/06.

Day zero: Defined as day of randomization. Study days are calendar days.

Drug held/hold drug: Study medication withheld for 24 hours.

Drug permanently discontinued: Study medication stopped for remainder of the trial.

Enrollment: All randomized participants will be included in the intent-to-treat (ITT) population. All randomized subjects who receive at least one dose of study product (CO vs. placebo) will be included in the safety analyses (*ie.* modified ITT). For secondary and exploratory endpoints, subjects may also be analyzed according to the number of study drug doses completed. Because some patients may withdraw or have a change in clinical status precluding dosing of study drug (eg. post- randomization lactic acidosis), we may randomize additional patients to achieve the enrollment goal of 36 patients who are randomized and treated with at least one dose of the study drug. We expect this to be an uncommon occurrence. Patients who are randomized but do not undergo any study procedures or study treatment will not be included in the study analysis.

Extubation: Removal of an orotracheal, nasotracheal tube, or unassisted breathing with a tracheostomy.

Home: level of residence or health care facility where the patient was residing prior to hospital admission

Hospital Mortality to Day 60: This primary endpoint includes all deaths following randomization in any health care facility prior to discharge “home” until study day 60. Study subjects still in a health care facility at study day 61 are considered alive for this endpoint.

Interruption of Dosing During Drug Administration: Study medication prematurely stopped prior to 90 minutes.

NYHA: New York Heart Association Class IV subjects (defined as subjects who have cardiac disease resulting in inability to carry out physical activity without discomfort. Symptoms of cardiac insufficiency or an anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased).

Pneumonia: Unilateral or bilateral pulmonary infiltrates in a patient with suspected respiratory infection.

Sepsis: Life-threatening organ dysfunction caused by a dysregulated host response to infection:

1. Suspected or proven infection: Sites of infection include thorax, urinary tract, abdomen, skin, sinuses, central venous catheters, and central nervous system.
2. Increase in SOFA score greater than or equal to 2 over baseline.

Study Drug: Defined as inhaled carbon monoxide or placebo.

Study hospital: Defined as the hospital where the patient was enrolled.

Study withdrawal: Defined as permanent withdrawal from study before completion of study activities.

This does not include those subjects who have completed the protocol procedures or stopped procedures because they have reached unassisted breathing. If a patient or surrogate requests withdrawal from the study, the investigators will seek explicit permission to continue data collection.

UAB (Unassisted Breathing): Spontaneously breathing with face mask, nasal prong oxygen, or room air, T-tube breathing, tracheostomy mask breathing, or CPAP ≤ 5 without PS or IMV assistance, or the use of noninvasive ventilation solely for sleep-disordered breathing. Assisted breathing is any level of ventilator support at pressures higher that the unassisted breathing.

2 INTRODUCTION

2.1 STUDY RATIONALE

The purpose of this study is to assess the safety and efficacy of an algorithm-specified dose of inhaled carbon monoxide (iCO) to achieve a target COHb level of 6-8% in mechanically ventilated patients with sepsis-induced ARDS. By studying subjects with ARDS, we have targeted a disease that has been well studied in rodent and non-human primate preclinical models. Furthermore, by focusing on intubated subjects with ARDS, we have chosen a group with higher disease burden and thus likely to have both increased mortality and an increased opportunity for benefit, including a reduction in the requirement for mechanical ventilation. In April 2023, we elected to expand inclusion criteria to include intubated patients with pneumonia (unilateral or bilateral pulmonary infiltrates consistent with lung infection), given reported similar outcomes in critically ill mechanically ventilated patients with unilateral and bilateral infiltrates (LUNG SAFE study).

Mitochondrial dysfunction is associated with increased disease severity and poor outcomes during sepsis and may be a key mechanism underlying ARDS and multiple organ dysfunction syndrome in critically ill patients[5]. Furthermore, early activation of mitochondrial biogenesis has been associated with improved survival in critical illness[6]. We and others have demonstrated that circulating mitochondrial DNA (mtDNA) levels are significantly increased in patients with sepsis and ARDS and correlate with mortality in critically ill patients[5,7,8]. We have also shown that CO can inhibit mitochondrial reactive oxygen species (ROS) generation, inhibit translocation of mtDNA into the cytosol, and preserve mitochondrial function in macrophages[9]. CO also has been shown to activate mitochondrial biogenesis in skeletal muscle in humans[10]. Taken together, these findings suggest that preservation of mitochondrial function may be a key mechanism by which CO protects against ARDS and multiple organ failure in critical illness.

2.2 BACKGROUND

2.2.1 INTRODUCTION

Acute respiratory distress syndrome (ARDS) is a syndrome of severe acute lung inflammation and hypoxemic respiratory failure with an incidence of 180,000 cases annually in the United States[11,12]. Despite recent advances in critical care management and lung protective ventilation strategies[13-16], ARDS morbidity and mortality remain unacceptably high[3,17,18]. Furthermore, no specific effective pharmacologic therapies currently exist. Sepsis, life-threatening organ dysfunction caused by a dysregulated host response to infection, represents a major risk for the development of ARDS and multi-organ dysfunction syndrome (MODS). In recent years, the number of patients with severe sepsis has risen to 750,000 per year in the U.S.[19-21], which bears an alarming forecast for critically ill patients in the intensive care unit with significant risk for the development of ARDS. The lack of specific effective therapies for pneumonia

and ARDS indicates a need for new treatments that target novel pathways. Pneumonia represents a lung infection with unilateral or bilateral infiltrates, and intubated patients with pneumonia not formally meeting ARDS criteria have been reported to have similar outcomes with unilateral or bilateral infiltrates. Pneumonia in intubated patients represents an important spectrum of illness in ARDS, and in fact the most recent NIH lung injury consortium to improve phenotyping in critical illness will enroll subjects with pneumonia, sepsis, and ARDS. Furthermore, our preliminary data demonstrating efficacy and safety of inhaled CO in unilateral pneumonia in baboons is presented below. Carbon monoxide (CO) represents a novel therapeutic modality in pneumonia and sepsis-induced ARDS based on data obtained in experimental models of pneumonia, sepsis, and ARDS over the past decade.

2.2.2 CO IS ENDOGENOUSLY PRODUCED GAS WITH PLEIOTROPIC BIOLOGICAL FUNCTIONS

CO is endogenously produced in the body by heme oxygenase-1 (HO-1), one of the few inducible molecules that can protect the lung from an increased oxidant burden under circumstances of stress[22]. HO-1 is ubiquitously expressed, and is responsible for degradation of heme to biliverdin, free iron, and CO. While all three products of its activity have been shown to possess cytoprotective properties, CO is the product that has been most extensively studied with respect to lung disease. This colorless, odorless diatomic gas has been shown to exert biological functions as diverse as protection against oxidative injury[23-25], inflammation[26], and cell death[27,28], inhibition of cell proliferation[29], suppression of matrix production[30], increased fibrinolysis[31], as well as enhanced phagocytosis[32,33] and macrophage efferocytosis[34], all of which may be important in the pathogenesis of sepsis and ARDS. Recently, we have demonstrated several mechanisms by which CO exerts these beneficial effects including activation of mitochondrial biogenesis[35-38], enhancement of autophagy[33,39,40], suppression of mitochondrial dysfunction and inflammasome activation[9], as well as acceleration of resolution of inflammation via production of specialized pro-resolving lipid mediators (SPMs)[34,38,41-43].

2.2.3 ADMINISTRATION OF INHALED CO PROTECTS AGAINST SEPSIS AND LUNG INJURY IN ANIMAL MODELS

Our laboratory and others have demonstrated that CO at a low physiological dose confers cytoprotection via potent anti-inflammatory, anti-apoptotic, and anti-proliferative effects[23-29]. These responses are regulated by cell- or tissue-specific, and stimuli- or stress-specific signaling pathways[44]. We and others worldwide have demonstrated that CO mediates cytoprotection in a variety of tissue injury models including experimental models of sepsis and acute lung injury (ALI) **(Table 1)**[24-26,28,30,32,33,38,45-55]. These studies have shown that low dose CO confers tissue protective effects in experimental ALI models including hyperoxia and endotoxin exposure, bleomycin, ischemia/reperfusion, and ventilator-induced lung injury (VILI)[24,25,30,45-50].

We have also recently shown that low dose iCO accelerates resolution of ALI in a clinically-relevant baboon model of pneumococcal pneumonia[38,41,42,56]. Administration of iCO at 200 ppm for 60 minutes at 48 hours following *S. pneumoniae* inoculation significantly attenuated histologic lung injury scores and reduced lung wet-to-dry ratios at 8 days[38]. CO-treated animals had significantly increased expression of citrate synthase and increased ATP synthase staining in alveolar type 2 cells and macrophages suggesting that CO induces mitochondrial biogenesis[38]. We also found that CO had systemic anti-oxidant effects with augmentation of SOD2 expression in the kidney, which co-localized with cytochrome *c* consistent with attenuated mitochondrial injury and cytochrome *c* retention in intact mitochondria[38]. CO also reduced pro-inflammatory urinary cysteinyl leukotrienes[42] and partially restored levels of circulating SPMs[41].

In addition, we and others have demonstrated that CO decreases inflammation, enhances phagocytosis, and improves mortality in models of sepsis including endotoxemia[26,51,52], hemorrhagic shock[53] and cecal ligation and puncture (CLP)[32,33]. Furthermore, CO has been shown to have beneficial therapeutic effects in pre-clinical models of other diseases including pulmonary hypertension[57-60], vascular injury[61-65], and transplantation[48,49,66-74] **(Table 1)**.

Table 1: Pre-clinical studies of inhaled CO

Model	Year	Species	CO (ppm)	Outcome	Reference
Lung injury (VILI, acid, hyperoxia, LPS, pneumonia)	1999, 2003, 2004, 2008, 2009, 2010, 2015	mouse, rat, macaques, baboon	10-250, 500	Less inflammation & lung injury	24,25,38,45-47,54
Bleomycin lung fibrosis	2005	Mouse	250	Decreased lung hydroxyproline, fibronectin, collagen	30
Ischemia-reperfusion (hind leg, lung)	2001, 2003, 2006, 2007, 2009	mouse, rat	250, 500, 1000	Less remote organ inflammation, less apoptosis, improved survival	28,31,50,75-77
Transplantation (liver, lung, kidney, heart, intestine)	2001, 2003, 2004, 2006, 2007, 2008, 2009	mouse, rat	20, 250, 400, 500	Improved survival & graft function, less inflammation & apoptosis	48,49,66-69,71-74,78
Endotoxemia	2000, 2003, 2004	rat, mouse	10-250	Improved survival, decreased inflammation	26,51,52
Hemorrhagic shock	2005	Mouse	250	Decreased end organ injury/ischemia	53
Cecal ligation and puncture	2008, 2014	Mouse	250	Improved survival, decreased inflammation, enhanced phagocytosis	32,33
Pulmonary arterial hypertension	2006	rat, mouse	50, 250	Reversal of established PAH & reversal of remodeling	57
Vascular injury	2003, 2005, 2006, 2007	mouse, pig	100, 250-500	less intimal hyperplasia, reduced thrombosis	61-65
Cardiopulmonary bypass	2004, 2008, 2009	Pig	250	Less lung injury, decreased cardiac edema, apoptosis	79-82

Model	Year	Species	CO (ppm)	Outcome	Reference
Doxorubicin cardiomyopathy	2007	Mouse	500	Improved cardiac function	83,84
Asthma	2003	Mouse	250-1000	Reduced inflammation & bronchoconstriction	30,85,86
Cerebral malaria	2007	Mouse	250	Reduced incidence of cerebral malaria	87
Hepatitis	2003, 2007	Mouse	100, 250, 500	Improved survival, decreased apoptosis	88,89
Colitis, ileus	2005	mouse, rat, pig	250	Reduced injury & inflammation, improved motility	57,90-92
Sickle cell disease	2009	Mouse	250	Reduced leukocytosis	93
Collagen-induced arthritis	2009	Mouse	200	Improved arthritis score, less inflammation	94
Ureteral obstruction	2008	Mouse	250	Reduced renal fibrosis	95

2.2.4 LUNG PROTECTIVE EFFECTS OF CARBON MONOXIDE

Numerous studies have examined the protective effects of low concentrations of CO on the pulmonary parenchyma and vasculature. Inhaled CO prolongs survival and prevents tissue injury and epithelial cell death in rodents subjected to high oxygen stress[24]. CO also reduces lung cell apoptosis during lung ischemia-reperfusion injury in mice[50] and prevents tissue injury during mechanical ventilation in mice by preventing alveolar-capillary barrier dysfunction and reducing inflammation[45-47]. Low concentration iCO can also reverse established pulmonary hypertension in rats[57] and has been shown to protect against endothelial apoptosis[27]. In addition, CO has been shown to de-repress fibrinolysis and to inhibit expression of plasminogen activator inhibitor-1, which could alter the progression of fibrosis[31]. CO has also been shown to confer protection in a number of additional disease models, including asthma, vascular injury, transplantation and fibrosis **(Table 1**). These studies collectively have provided a rationale for pursuing the clinical applications of CO, including the trials outlined below.

2.2.5 CO DELIVERY VIA AN INHALED ROUTE IS SAFE IN HUMAN SUBJECTS

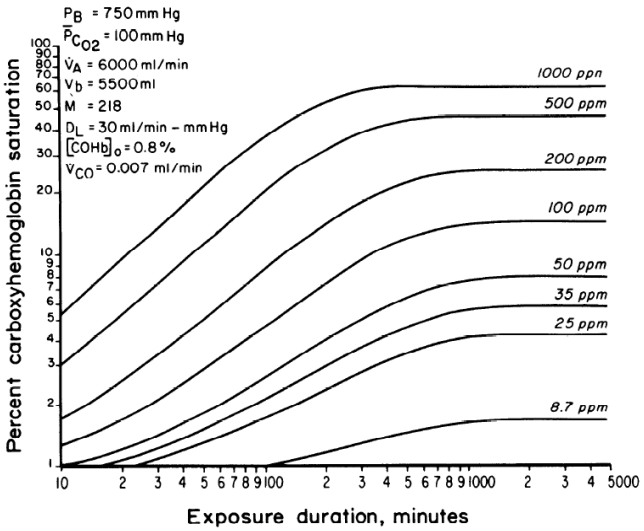


Figure 1. COHb levels as a function of exposure duration and CO concentration as determined by the CFK equation. (Peterson et al., 1975)

CO has proven to be an ideal gas for developing theoretical uptake equations. The formation of carboxyhemoglobin (COHb) on the basis of CO exposure is well described by a physiologically based pharmacokinetics model developed by Coburn in 1965 and is referred to as the **Coburn-Foster-Kane equation** (typically identified as the CFK or CFKE) in the literature[96]. This model has been tested and confirmed in humans for varying inspired CO concentrations and durations of exposure[97-103] **(Figure 1)**.

Extensive data is available regarding the safety and tolerability of low dose iCO in healthy volunteers[10,101,104-108] and more recently, in subjects with chronic obstructive pulmonary disease (COPD)[109] and idiopathic pulmonary fibrosis (IPF) **(Table 2)**. Previous studies have carefully measured COHb levels in response to iCO and demonstrated that low dose iCO is safe in healthy normal volunteers[10,35,101,104-108]. Stewart et al. performed 25 exposures to known CO concentrations in healthy volunteers; in this study, exposure to 100 ppm CO for 8 hours resulted in COHb levels of 11-13% with no adverse effects in time estimation, steadiness, manual dexterity, EEG, and evoked potentials[106]. In a study aiming to simulate cigarette smoke inhalation, Zevin et al. exposed healthy volunteers to iCO (1200-1500 ppm) for 10 minutes, repeating every 45 minutes for 16 hours per day for 7 days[107]. In this study, mean COHb levels were 5 ± 1% and no adverse events (AEs) were reported[107].

Pecorella *et al*. has also demonstrated safety (COHb 5.4 ± 0.79%) and activation of mitochondrial biogenesis in healthy individuals after CO inhalation at 200 ppm for 1 hour per day for 5 consecutive days[35].

Table 2: Clinical Trials of CO in Human Subjects

Study	CO Exposure	COHb	Adverse Effects
Stewart (1970)[106]	100 ppm for 8 h	11-13%	No adverse effects
Peterson (1975)[101]	50-200 ppm, up to 5.25 h	1-20%	None noted
Hausberg (1997)[104]	1000 ppm x 30 min, then 100 ppm x 30 min	8.3 ± 0.5%	None reported
Zevin (2001)[107]	1500 ppm x 10 min, then every 45 min x 16 h for 7 days	5 ± 1%	None reported
Ren (2001)[108]	4000 ppm until COHb ~ 10%, then repeated to keep COHb ~ 10% for 8 h	9.7 ± 0.1%	None reported
Mayr (2005)[105]	500 ppm for 1 h	6.5-7.7%	Mild headache in 1 subject

Rhodes (2009)[10]	100 ppm for 1 h for 5 days	3.3 ± 0.6%	No adverse effects
Pecorella (2015)[35]	200 ppm for 1 h for 5 days	5.4 ± 0.79%	No adverse effects
Bathorn (2007)[109]	125 ppm for 2 h, 4 consecutive days (COPD)	2.1-3.4%	2 COPD exacerbations, judged unrelated
NCT00094406 (IND# 70,694)	100 ppm for 6 h	6.5 ± 1.7%	No adverse effects
Rosas (2017)[110]	100-200 ppm x 2 h, 2 times weekly x 12 wks (IPF)	3-4%	Well tolerated, no SAEs related to CO
Fredenburgh (2018)[111,112]	100 or 200 ppm for 90 min for up to 5 days	3.48 ± 0.7% (100 ppm) 4.9 ± 0.28% (200 ppm)	Well tolerated, no SAEs related to CO

In a study evaluating the effects of hypoxemia, hemodilution, and carboxyhemoglobinemia on respiratory control, Ren et al. exposed 11 normal volunteers to an iCO treatment regimen aiming to maintain a COHb level of 10% for 8 hours[108]. COHb levels ranged from 9.1 to 10.5% (mean 9.7%) and no AEs were reported[108]. Similar results have been published in a number of other studies, and none have reported AEs[10,35,101,104,105]. In fact, baseline COHb levels of 3% have been reported in some urban areas[106] and levels as high as 10-15% may be observed in asymptomatic chronic smokers[113-115].

We have conducted three studies, one in normal human subjects (NCT00094406), one in subjects with IPF (NCT01214187)[110], and one recently completed Phase I trial in sepsis-induced ARDS (NCT02425579)[112]. In our placebo-controlled study of 24 healthy volunteers (11 females; mean age 26.2 ± 5.2 yrs), individuals were randomized to treatment with room air or iCO (100 ppm) for 6 hours following endotoxin instillation. CO treatment was well tolerated and mean COHb levels were 6.5% ± 1.7% in CO-treated subjects. There were no significant differences in vital signs, neurocognitive studies (including immediate and delayed memory, attention, language and visuospatial/constructional function), or AEs **(Table 3)**.

Table 3: Vital Signs and Laboratory Parameters in CO-Treated Healthy Volunteers.

Parameter	Room Air	CO (100 ppm)	p value
Respiratory rate	17.7 ± 3.6	16.7 ± 4.0	0.5
Heart rate	84.5 ± 14	83.8 ± 15	0.9
Temperature °C	36.9 ± 0.4	37.2 ± 0.4	0.15
Mean arterial pressure (mm Hg)	92.8 ± 11	98.7 ± 11	0.21
Carboxyhemoglobin (%)	1.1 ± 0.7	6.5 ± 1.7	0.001
Oxyhemoglobin (%)	96.3 ± 2.1	92.3 ± 1.9	0.002
PaO_2	96.5 ± 11	94 ± 7.3	0.54
Arterial pH	7.4 ± 0.02	7.41 ± 0.02	0.29
Lactate (mmol/L)	0.7 ± 0.26	0.62 ± 0.19	0.41

In addition to studies in healthy volunteers, prior work has demonstrated the feasibility of administering low dose iCO to subjects with COPD[109]. In this study, ex-smoking subjects with stable COPD were treated with iCO at 100-125 ppm for 2 hours per day on 4 consecutive days. This led to COHb levels of 2.1-3.4% with a maximal individual COHb level of 4.5%. Inhalation of CO by subjects with stable COPD led to trends in reduction of sputum eosinophils and improvement in methacholine responsiveness[109]. In our recently completed 8-center Phase I/II trial of low dose iCO in IPF[110,116], 58 subjects were randomized to treatment with iCO (100-200 ppm) or 21% O_2 for 2 hours, twice weekly for 12 weeks. Inhaled CO was well-tolerated

with no significant difference in AEs or neurocognitive function by the Montreal Cognitive Assessment (MoCA) between iCO-treated and control subjects[110].

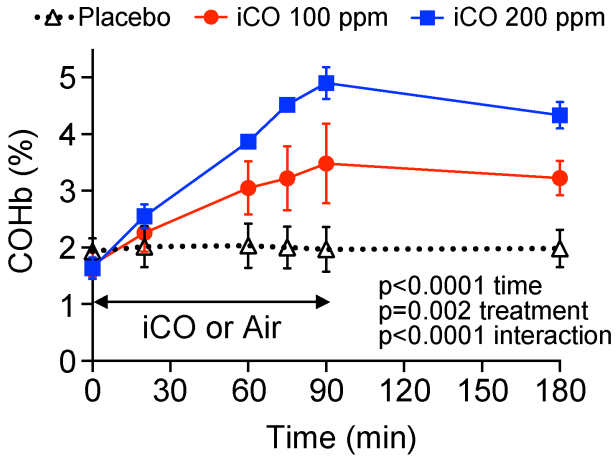


Figure 2. Low dose iCO can be safely and accurately administered in mechanically ventilated subjects with ARDS.

More recently, we completed a Phase I trial of low fixed-dose iCO in patients with sepsis-induced ARDS[111,112]. Twelve participants were randomized to iCO or placebo air 2:1 in two cohorts. Four subjects each were administered iCO (100 ppm in Cohort 1 or 200 ppm in Cohort 2) or placebo for 90 minutes for up to 5 consecutive days. CO treatment was well tolerated and no participants exceeded a COHb level of 10%. There were no administration-associated AEs or study-related serious AEs (SAEs). Baseline COHb levels were not different across treatment groups. CO-treated participants had a significant increase in COHb (3.48 ± 0.7% [Cohort 1]; 4.9 ± 0.28% [Cohort 2]) compared with placebo-treated subjects (1.97 ± 0.39%), which did not differ from baseline levels **(Figure 2)**. The maximum COHb level in Cohort 1 was 4.4% and 6.87% in Cohort 2. The maximum change in COHb from baseline to 90 minutes (Δ 90 min COHb) was 2.5% in Cohort 1 and 4% in Cohort 2. There was no significant increase in COHb in placebo subjects.

Taken together, these findings demonstrate that experimental administration of several different concentrations of CO is well tolerated and that low dose iCO can be safely administered to subjects in a controlled research environment.

2.2.6 STEADY STATE DIFFUSING CAPACITY AND THE SAFETY OF INHALED CARBON MONOXIDE

CO is also a diagnostic gas that has been used for more than a century to evaluate lung function, and in particular, in the steady state diffusing capacity test to determine the function of the alveolar-capillary membrane. The steady state method of measuring the diffusing capacity of the lung dates back more than 100 years, based on the research of Haldane and Smith. In the early 1900s, Krogh and Barcroft developed it into a standard test procedure for both understanding alveolar membrane function, as well as for diagnosing diseases of the alveolar-capillary membrane. Up until the 1970s, steady state diffusing capacity was the standard diagnostic test for pulmonary laboratories worldwide, at which point it was replaced by the single breath diffusing capacity test due to its better accuracy and being less time consuming.

The steady state diffusing capacity test is still used today in some instances where the single breath procedure is not as practical, such as measurements during exercise. The procedure for steady state diffusing capacity testing entails patients inhaling 0.1% CO (1000 ppm) for seven minutes[117]. As duplicate or triplicate measurements are required for most lung function tests, this suggests that hundreds of thousands of people have inhaled 1000 ppm for 14 to 21 minutes with no known reports of adverse events associated with the test. Based on the curves from the CFK equation **(Figure 1)**, it is likely that this same number of people had their COHb levels raised above 3-6% following the diagnostic procedure. This longstanding diagnostic procedure reinforces that inhaling a constant concentration of low dose CO can be safely done without significant adverse events.

2.2.7 DELIVERY OF INHALED CO TO MECHANICALLY VENTILATED SUBJECTS

Figure 3. CO Delivery System (12th Man

In order to study inhaled CO in mechanically ventilated subjects with pneumonia and sepsis-induced ARDS, a Carbon Monoxide Delivery System (COventDS) **(Figure 3)** developed by 12th Man Technologies will be used **(Appendix A- CO Delivery System)**. The CO Delivery System is a microprocessor-based constant gas concentration delivery system that can be used to deliver operator-specified concentrations of CO to mechanically ventilated patients. The CO Delivery System delivers an operator set, constant concentration of CO gas into the inspiratory path of the patient breathing circuit independent of the patient's inspiratory flow, while the patient's respiration is supported by a ventilator. For safety reasons, the CO Delivery System has twin microprocessors such that the division of control is split between a closed loop proportional-integral-derivative (PID) controlled mixing module that is only involved with monitoring the patient flow and mixing of the gases, and an interface module that is the working face to the user for control and monitoring functions. This second microprocessor watches the delivery subsystem, monitors the inspired gas for deviations from the set concentration, and monitors inspired oxygen.

The heart of the system is comprised of three components including an inspiratory flow monitor with a ratio-metric matching CO injector module, an inhaled gas monitoring module, and a gas mixing subject interface/breathing valve for spontaneously breathing subjects. It is a variable inspiratory flow delivery system that matches the patient's inspiratory flow with the injected 5000 ppm CO to deliver the operator set CO concentration on the LCD user interface. It is a breathing-initiated delivery system and the CO is blended into the inspiratory gas stream only as long as flow is being delivered to the patient and at the exact proportions to maintain the desired concentration, independent of any change in breathing pattern, flow rate, respiratory rate, or tidal volume. The analyzer, with alarm functions, monitors the inhaled CO and O_2 concentrations.

The CO Injector is a core component for the delivery of CO. The CO Injector is constructed of CO-compatible materials and consists of a pressure regulation circuit that reduces the 40-60 psig inlet CO gas source to the optimal pressure for its proportional flow control valve. Upon sensing inspiratory flow by the patient with the flow monitoring interface, the injector module will track the flow and match the volume of CO injected to the volume of inspired gas to keep the concentration of CO constant independent of the patient's pattern of inspiratory flow. The PID controlled mixing module's sole function is to read the patient's inspiratory flow and inject CO proportional to that flow in 1 millisecond intervals. Alarms will sound on high or low CO or O_2 concentrations. Should the inspired CO concentration rise above 660 ppm, in addition to the alarms, the system will stop injecting CO into the circuit.

2.2.8 TESTING THE CO DELIVERY SYSTEM IN BABOON MODEL OF PNEUMOCCAL PNEUMONIA

We evaluated the safety and efficacy of the CO Delivery System developed by 12th Man Technologies in a baboon model of S. pneumoniae pneumonia **(Figure 4)**. Five juvenile, male colony-bred baboons (Papio cynocephalus) were intubated, sedated, and mechanically ventilated[38]. The animals underwent bronchoscopy and a baseline bronchoalveolar lavage (BAL) was performed followed by instillation of Streptococcus pneumoniae (10^8 - 10^9 CFU) in the right and left lower lung zones. At 24 or 48 hours post-inoculation, animals were sedated, intubated, ventilated, and underwent a repeat bronchoscopy and BAL. The CO Delivery System was calibrated using 100 ppm and 400 ppm CO tanks and readied for use with a

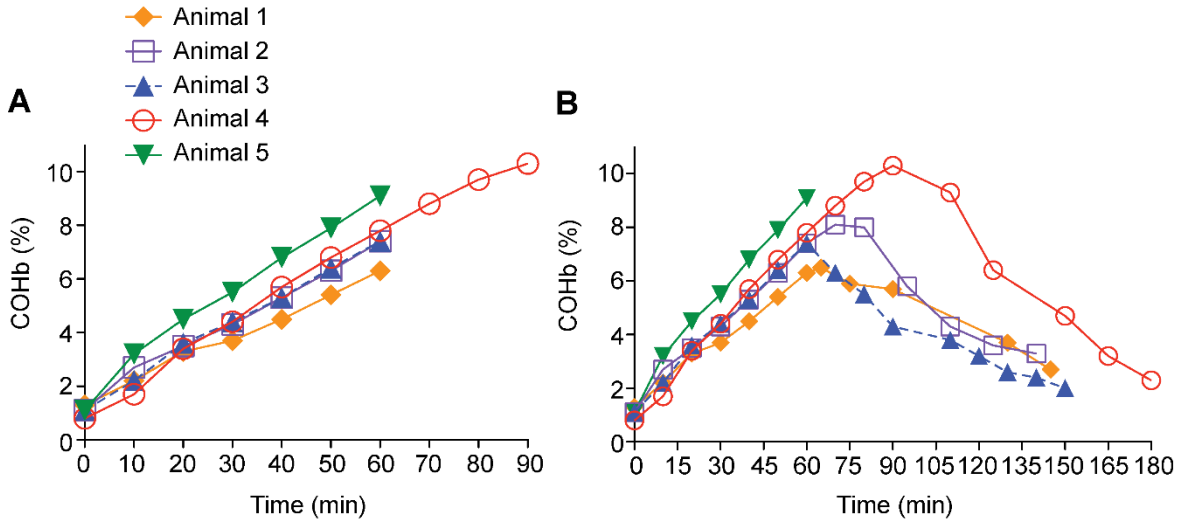


Figure 4. Delivery of inhaled CO at 200 ppm and COHb levels in baboon pneumonia model.

5000 ppm CO source cylinder. All CO tanks contained CO gas at the specified concentration in air. Ambient CO levels were monitored during the CO delivery device assembly, calibration, and continuously throughout the experiment using a CO detector.

Following bronchoscopy, animals were administered iCO at 200 ppm through the ventilator via the CO Delivery System for 60-90 minutes. After iCO treatment was completed, animals were administered supplemental FiO_2 for 60-90 minutes until COHb levels returned to near baseline levels. Arterial blood was drawn before, during, and after CO delivery at 10-15 minute intervals and arterial blood gas (ABG) and COHb measurements were performed. In certain experiments, both venous and arterial blood samples were drawn simultaneously for measurement of venous and arterial COHb levels respectively **(Figure 5)**. COHb levels were measured using the IL 682 Co-oximeter and, in certain experiments, using the AVOXimeter 4000 Co-oximeter. After CO exposure, animals were administered ceftriaxone daily for a total of 3 days.

After one hour of iCO administration at 200 ppm, animals achieved the pre-specified goal COHb level of 6-8%. At baseline, arterial COHb levels were 1.1 ± 0.2% and increased linearly to 2.4 ± 0.6%, 3.7 ± 0.5%, 4.5 ± 0.7%, 5.5 ± 0.8%, 6.6 ± 0.9%, and 7.6 ± 1% at 10, 20, 30, 40, 50 and 60 minutes of iCO administration[38], respectively (p<0.0001) **(Figure 4A)**. One animal was intentionally given a prolonged (90 minute) exposure which similarly demonstrated a linear rise in COHb to 7.8%, 8.8%, 9.7%, and 10.3% at 60, 70, 80, and 90 minutes, respectively. Peak COHb levels decreased following administration of FiO_2 1.0, returning to near baseline levels after 82 ± 9.5 minutes[38] **(Figure 4B)**. Throughout the exposure, ambient CO levels remained ≤1 ppm. Low dose iCO treatment was well-tolerated with no significant differences in pre- and post-CO heart rate, blood pressure, temperature, PaO_2, or minute ventilation.

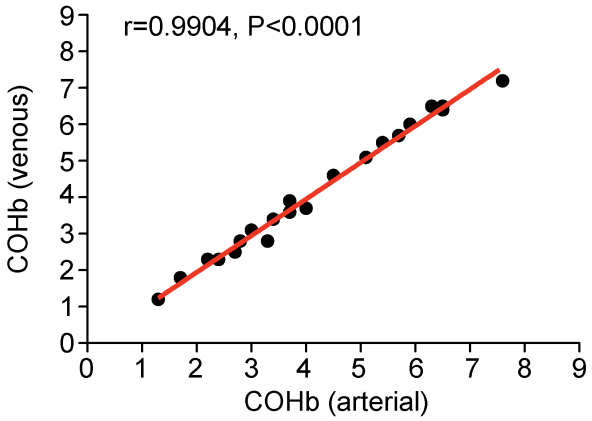


Figure 5. Correlation between arterial and venous COHb levels.

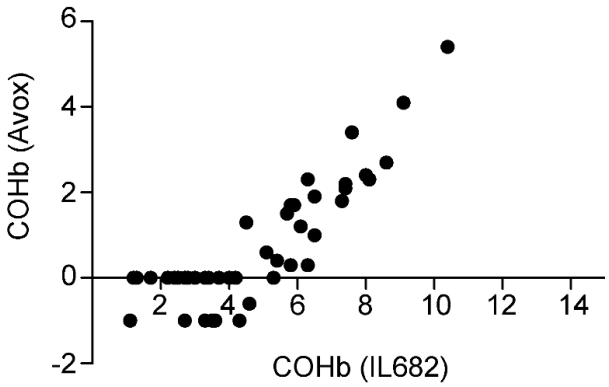


Figure 6. Measurement of COHb levels by AVOX4000 versus IL682 Co-oximeters.

Using the IL 682 Co-oximeter, we found that venous and arterial COHb levels were highly correlated (r=0.9904, p<0.0001)[38]. Modeling these data with type II linear regression, we found that the regression line was a near-perfect diagonal with a slope of 1.003 (95% CI [0.952 – 1.05]) and a y-intercept of -0.064 (95% CI [-0.2941 – 0.1661])[38] **(Figure 5)**. This tight correlation argues that venous measurements are as accurate and reliable as arterial measurements. To determine the accuracy and precision of the point-of-care AVOXimeter 4000 Co-oximeter relative to the gold-standard IL 682 Co-oximeter, we measured COHb levels using both devices on synchronously drawn arterial blood. Compared with the IL 682 Co-Oximeter, the AVOXimeter 4000 Co-oximeter was substantially less sensitive as COHb was not detected until levels had reached 4-5%. Furthermore, the AVOXimeter consistently reported COHb levels that were four percentage points lower than the gold standard **(Figure 6)**.

Ambient CO levels in the experiment room remained at 0-1 ppm during assembly, calibration, and use of the CO delivery device. These levels are well below the OSHA permissible exposure limit of 50 ppm as an 8 hour time-weighted average (U.S. Department of Labor, Occupational Safety & Health Administration, https://www.osha.gov/chemicaldata/chemResult.html?RecNo=462).

2.2.9 CO DOSING STRATEGY USING CFK EQUATION

In order to develop a safe and effective dosing strategy based on an initial short exposure to inhaled CO, we also evaluated the accuracy of the **Coburn-Forster-Kane (CFK) equation (below)**[96,101] to predict COHb levels in baboons using measured COHb levels following a 10 and 20 minute CO exposure. Using the 10 minute COHb, we found that there was good correlation between measured COHb levels and the COHb levels predicted

$$\frac{A[\mathrm{HbCO}]_t - B\dot{V}\mathrm{co} - \mathrm{P_{I_{CO}}}}{A[\mathrm{HbCO}]_0 - B\dot{V}\mathrm{co} - \mathrm{P_{I_{CO}}}} = \exp\left(-tA/\mathrm{Vb}B\right)$$

by the CFK equation (r=0.9038, p<0.0001)[38]. However, there was superior correlation between measured COHb levels and COHb levels predicted by the CFK equation using the 20 minute COHb (r=0.9828, p<0.0001)[38] **(Figure 7A)**.

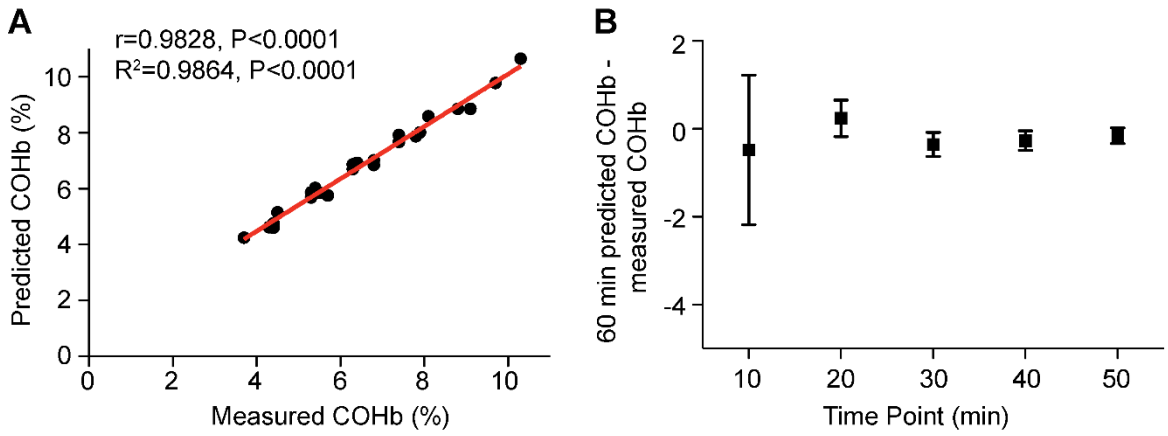


Figure 7. Exposure to CO for 20 min allows for accurate prediction of 60 min COHb using CFK equation.

Modeling these data with linear regression, we found that the regression line was a near-perfect diagonal with a slope of 0.9373 (95% CI [0.8885 – 0.9860]), a y-intercept of 0.7291 (95% CI [0.3976 – 1.061]), and goodness-of-fit R^2=0.9864 (p<0.0001)[38]. Furthermore, the 20-minute measured COHb was highly accurate in predicting the 60 minute COHb with a difference between predicted and measured COHb of 0.24 ± 0.33% (95% CI [-0.17 – 0.66])[38] **(Figure 7B)**. Taken together, by inputting the 20-minute measured COHb level into the CFK equation, we can predict the COHb level after 60 minutes of CO exposure with high accuracy.

In our recently completed Phase I fixed dose iCO trial, we also found an excellent correlation between measured and predicted COHb levels using the CFK equation[112]. While the CFK equation is a well-validated model of CO uptake that accurately predicts the rise in COHb following CO exposure in humans with normal lung function[97-103], it had not been previously used in ARDS patients with impaired gas exchange before. We found an excellent correlation in participants treated with 100 ppm iCO (Spearman r= 0.8614; p<0.0001), but an even stronger correlation in subjects treated with 200 ppm iCO (Spearman r=0.916; p<0.0001) **(Figure 8A)**. Modeling the 200 ppm Cohort 2 data with linear regression revealed a slope of 1.112 (95% confidence interval [CI] [1.003, 1.22]), a y-intercept of –0.6632 (95% CI [–1.174, –0.1522]), and goodness-of-fit R^2=0.9204 (p<0.0001). Bland-Altman plots also demonstrated excellent agreement between measured and CFK equation-predicted COHb levels **(Figure 8B)** with a mean difference between measured and predicted COHb levels of 0.1467 ± 0.2738 in Cohort 2 (200 ppm).

The above predictions using the CFK equation were made using a MATLAB-generated computer program to estimate DLCO using the baseline and 20 minute COHb. The estimated DLCO was then input into the programmed CFK equation and used to predict the 60, 75, and 90 minute COHb levels. This program was validated by generating the previous published curves **(Figure 1)** that were derived from predicted values using the CFK equation[101].

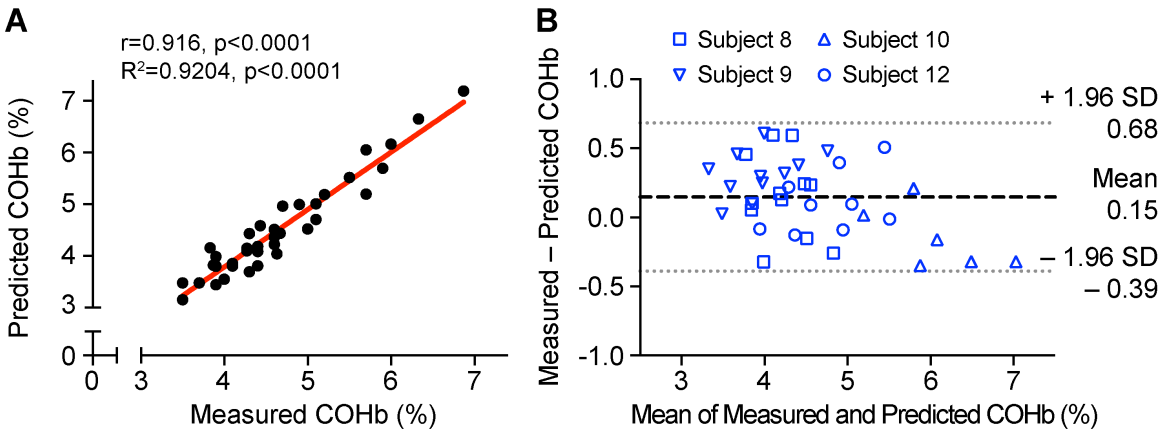


Figure 8. CFK equation accurately predicts COHb levels in ARDS patients.

CFK Equation For Precision-Based iCO Dosing

Our Phase I fixed-dose iCO study showed that precise delivery of low dose iCO is feasible and safe in mechanically ventilated ARDS patients. However, as anticipated, patients with ARDS of varying severity had different degrees of CO uptake and did not achieve the target COHb level of 6-8% with fixed iCO dosing, as was observed in our baboon ALI model. These results were predictable, based on key variables in the CFK equation that influence CO uptake. These data suggest that patients with ARDS require a personalized approach to iCO dosing to ensure optimal, consistent, and safe CO uptake in the setting of variable gas exchange. To estimate the range of iCO doses that may be required in ARDS patients, we used the CFK equation to predict COHb levels for a range of CO concentrations and exposure times using mean values for DL_{CO}, V_A, weight[118-123], Hgb, and FiO_2 in patients with ARDS **(Table 4)**. This modeling demonstrated that the average ARDS subject will likely require doses in the range of 200-500 ppm to achieve a target COHb level of 6-8%.

Table 4. Predicted COHb levels in ARDS.

CO (ppm)	10 min	20 min	30 min	40 min	50 min	60 min	90 min	120 min
200	1.2	1.6	2.0	2.3	2.7	3.0	3.8	4.5
300	1.4	2.1	2.6	3.2	3.7	4.2	5.4	6.5
400	1.7	2.5	3.3	4.0	4.7	5.3	7.0	8.4
500	1.9	2.9	3.9	4.8	5.7	6.5	8.6	10.3
600	2.1	3.4	4.6	5.7	6.7	7.7	10.2	12.2
1000	3.0	5.2	7.1	9.0	10.7	12.3	16.3	19.4

Mean ARDS values: DLCO 3.45 mL/min/mmHg, V_A 4.35 L/min, weight 77.1 kg, Hgb 8.5 g/dL, FiO_2 0.6.

Given the severe diffusion impairment and variable degree of CO uptake in patients with ARDS, we have developed an iCO dosing strategy using the CFK equation to personalize iCO dosing based on each patient's unique lung physiology. Our prior Phase I fixed dose iCO trial results show that the CFK equation is highly accurate at predicting COHb levels, suggesting that the CFK equation can be used in a novel personalized medicine approach to determine in real-time an optimal and safe iCO dose to achieve a target COHb level of 6-8% in ARDS patients with varying degrees of impaired gas exchange. This randomized, partially double-blind, placebo-controlled Phase Ib trial will evaluate the safety and accuracy of a novel CFK equation-based personalized iCO dosing approach in patients with sepsis-induced ARDS.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Potential risks of active study drug include headache and tachycardia. In cases of overdose, patients can have nausea, vomiting, seizures, problems thinking, coma, cardiopulmonary arrest, and death. These adverse effects are seen at doses much higher than those proposed in this study. Subjects will be vigilantly monitored for side effects during study drug administration and COHb and lactate levels will be carefully monitored throughout the study. There may be other risks of iCO in patients with ARDS that are currently unknown. Subjects will be monitored closely throughout their participation in the trial. In our recently completed Phase I trial, no protocol-specified iCO administration-related AEs occurred in 12 subjects, and the Data and Safety Monitoring Board (DSMB) has recommended moving forward with Phase Ib and Phase II studies after three interim analyses.

Although there is a risk of toxicity associated with inhalation of high concentrations of CO, low dose iCO has been shown to be protective in animal models and safe in human subjects. Data from animal studies demonstrate that iCO has beneficial effects on outcomes in sepsis and ALI. Furthermore, extensive data is available regarding the safety and tolerability of low dose iCO in healthy volunteers and more recently, in subjects with COPD and IPF. Subjects enrolled in the study will receive the same standard of care that is otherwise available to non-study patients in an ICU setting.

Risks of blood draws
All subjects will have blood drawn for research purposes. Most blood will be drawn through existing indwelling catheters. Risks of drawing blood percutaneously are uncommon and include bleeding and bruising.

Risks of radiation exposure
Most chest X-rays will be performed daily as part of usual ICU care in intubated patients. However, if not performed, we will obtain chest X-rays for endpoint assessment on study days 1-5 and study day 7. The risk of ionization radiation from routine chest X-rays is generally considered minimal due to the low dose (~0.1 milliSievert [mSv]).

Risks to privacy and/or confidentiality
There is a very low risk of loss of confidentiality but this should be mitigated by the exclusive use of coded samples. All study personnel with access to samples or data will be required to undergo HIPAA and CITI training as per institutional policies. Subject confidentiality will be protected throughout the study and no subject-identifying information will be released to anyone outside the study. Confidentiality will be maintained as detailed above.

Subjects will not be identified by name in any reports or publications, nor will the data be presented in such a way that the identity of individual subjects can be inferred. Analysis files created for further study by the scientific community will have no subject identifiers. Clinical information will not be released without the written permission of the subject, except as necessary for monitoring by the Clinical Coordinating Center (CCC), Data Coordinating Center (DCC), single IRB (sIRB), NHLBI, FDA or other authorized federal agencies. We will use methods that are scientifically rigorous and valid and in accordance with GCP guidelines. All research personnel have completed training in the Responsible Conduct of Research and the Protection of Human Research Subjects including GCP training.

2.3.2 KNOWN POTENTIAL BENEFITS

Study subjects may or may not receive any direct benefits from their participation in this study. Our preclinical data demonstrate that low dose iCO is protective in experimental models of sepsis and ALI.

Numerous studies have shown CO to be cytoprotective in animal models of lung injury including hyperoxia and endotoxin exposure, bleomycin, ischemia/reperfusion, pneumococcal pneumonia, and VILI[24,25,30,45-50,54]. Furthermore, CO has been shown to decrease inflammation, enhance phagocytosis, and improve mortality in models of sepsis including endotoxemia[26,51,124], hemorrhagic shock[53,125-127], and CLP[32,33,43]. In addition, CO has been shown to have beneficial therapeutic effects in pre-clinical models of other diseases including traumatic brain injury (TBI)[128], kidney fibrosis[95], colitis[90,92], arthritis[94], organ transplantation[48,49,66-74], hepatitis[88,89], and vasculopathies of the heart and lung[57,62-64,84].

Potential benefits from the administration of iCO include decreased requirement for ventilatory support, decreased days spent in the ICU, increased organ failure free days, and enhanced survival.

In addition to the potential benefit of iCO administration, all subjects, including those randomized to placebo, will benefit from protocolized low tidal volume ventilation and weaning protocols, as these have been shown to be beneficial in prior studies and are recommended in evidence-based consensus guidelines as best practice. As this protocol incorporates best practice guidelines, participation in the study will reduce the unexplained or potentially harmful variability in the application of such guidelines, and thus likely improve care, for all participants in our trial, including those participants randomized to placebo. Furthermore, there may be salutary effects on all participants given the additional clinical personnel and monitoring that will occur during administration of the study products.

Finally, there are potential benefits to society as the discovery of effective therapies that can reduce the substantial morbidity and mortality of ARDS or the identification of biomarkers that might improve prediction and monitoring of ARDS disease severity would enhance the health of society.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Federal regulations at 45 CFR 46. 111 (a)(2) require that “the risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result.” There are several elements of study design in the present protocol that meets this human subject protection requirement. Several of the exclusion criteria prohibit participation of patients who might be at increased risk from the effects of iCO. These include individuals with severe hypoxemia, acute myocardial infarction (MI), stroke within 1 month, cardiac arrest requiring CPR within 72 hours, or inability to assess mental status following cardiac arrest, angina pectoris with activities of daily living, cardiopulmonary disease (NYHA class IV), as well as women who are pregnant or breastfeeding. In addition, to ensure the safety of subjects and minimize potential risks related to the study drug administration, our study protocol includes stringent monitoring of subjects, provisions for daily hold parameters, criteria for interruption of dosing, and permanent discontinuation of the study drug.

Based on the preceding assessment of risks and potential benefits, the risks to subjects are reasonable in relation to anticipated benefits, and commensurate with those inherent in the subjects' expected critical illness requiring ICU care. There are currently no alternative available approaches to treat intubated patients with pneumonia or ARDS itself, besides supportive care, treatment of the underlying disease (*eg.* infection), and lung protective ventilator strategies. In addition, there are currently no biomarkers to assess disease severity and outcomes in pneumonia or ARDS. The potential risks of participation include those related to blood draws, chest X-rays, and those related to iCO treatment.

CO Treatment: Although there is a risk of toxicity associated with inhalation of high concentrations of CO, low dose iCO has been shown to be protective in animal models and safe in humans. No protocol-specified

iCO administration-related adverse events occurred in 12 subjects in our prior Phase I fixed-dose iCO trial, and the DSMB recommended advancing iCO to Phase Ib and Phase II sepsis and ARDS studies after three interim analyses. Data from animal studies demonstrate that iCO has beneficial effects on outcomes in sepsis and ALI. There is potential benefit to society and individual patients should iCO treatment prove to be beneficial for future patients with pneumonia, sepsis, and ARDS.

Subjects will be vigilantly monitored for side effects during drug administration and COHb and lactate levels will be carefully monitored as outlined. There may be other risks of iCO in patients with ARDS that are currently unknown. Subjects will be monitored closely throughout their participation in the trial.

Blood draws: All enrolled subjects will have blood drawn for both safety monitoring and research purposes. Most blood will be drawn through indwelling catheters. Risks of drawing blood percutaneously are uncommon and include bleeding and bruising. The risks associated with this common clinical practice are small, whereas the knowledge gained in furthering our understanding of ARDS and development of a potential new therapy for patients with ARDS may be substantial.

Radiation Exposure: Most chest X-rays will be performed as part of usual ICU care. The risk of ionization radiation from routine chest X-rays is generally considered minimal due to the low dose (~0.1 milliSievert [mSv]).

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To evaluate the safety and accuracy of a CFK equation-based personalized iCO dosing algorithm in patients with pneumonia and/or sepsis-induced ARDS.	**1. Safety** of iCO, defined by the incidence of pre-specified administration-related AEs and SAEs. Administration-related SAEs are: 1) myocardial infarction; 2) stroke; 3) new onset arrhythmia requiring DC cardioversion; 4) worsening hypoxemia[1,2]; 5) COHb ≥ 10%; and 6) increase in lactate by ≥ 2 mmol/L. **2. Accuracy** of the CFK equation-based personalized iCO dosing algorithm. This will be assessed by comparing the measured COHb level at 90 minutes and the target COHb level of 6-8%.	**1. Safety** of iCO: These administration-related SAEs were used in our prior Phase I fixed-dose iCO trial and are SAEs that could potentially occur if there were decreased delivery of oxygen. A COHb level ≥ 10% was chosen as an administration-related SAE as the goal COHb is 6-8%. **2. Accuracy** of personalized iCO dosing approach: The accuracy of the iCO dosing algorithm to achieve the target COHb level (6-8%) will be evaluated.
Secondary		
To determine the effect of low dose iCO therapy on clinical outcomes in patients with	1. Lung Injury Score (LIS) on days 1-5 and day 7 2. PaO2/FiO2 on days 1-5 and day 7	LIS, PaO_2/FiO_2, OI, and Vd/Vt have been shown to correlate with VFDs and

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
pneumonia and/or sepsis-induced ARDS.	3. Oxygenation index (OI) on days 1-5 and day 7 4. Dead space fraction (Vd/Vt) on days 1-3 and day 7 5. Sequential Organ Failure Assessment (SOFA) scores on days 1-5, 7, 14, and 28 6. Ventilator-free days (VFDs) to day 28 7. ICU-free days to day 28 8. Hospital-free days to day 60 9. Hospital mortality to days 28 and 60 10. Neurocognitive function at 3 and 6 months	mortality in prior ARDS studies.
Exploratory		
To examine the effects of low dose iCO therapy on biologic readouts in patients with pneumonia and/or sepsis-induced ARDS.	Blood and urine biomarkers of mitochondrial dysfunction (mtDNA), inflammasome activation (IL-18) and necroptosis (RIPK3). Lipid mediators (LM) and specialized pro-resolving mediators (SPMs).	Our preliminary data suggest that CO may modulate these biomarkers.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a multi-center, randomized, partially double-blind, and placebo-controlled **Phase Ib safety clinical trial** of inhaled CO (iCO) for the treatment of pneumonia and/or sepsis-induced ARDS. We hypothesize that low dose iCO will be safe and well-tolerated and that the CFK equation-based iCO personalized dosing algorithm will be accurate in achieving a target COHb level of 6-8% in patients with pneumonia and/or sepsis-induced ARDS.

Intubated subjects (n=36) with pneumonia and/or sepsis-induced ARDS who have consented to participate will be randomized in a 2:1 ratio to treatment with iCO (n=24) or medical grade air (n=12) for 90 minutes daily for 3 consecutive days. Subjects will be administered iCO or placebo air via inhalation through the mechanical ventilator using the CO Delivery System (COventDS, 12th Man Technologies). Subjects randomized to CO will be administered iCO at an individualized dose between 200-500 ppm as determined by a personalized iCO dosing algorithm to achieve a COHb level of 6-8%. Subjects randomized to placebo will receive medical grade air through the COventDS from identical appearing gas cylinders. The study drug will be administered for up to 3 days following randomization or until discontinuation of mechanical ventilation, whichever occurs first. For patients who have a tracheostomy, the equivalent of extubation for the purposes of this protocol will be breathing via tracheostomy with unassisted breathing.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Low dose iCO has been shown to be safe and well-tolerated in Phase I/II trials[10,35,101,104-110]. Our group has developed and tested a ventilator-compatible CO Delivery System and a CO dosing strategy in a non-

human primate model of pneumonia-induced ALI[38]. We demonstrated that low dose iCO can be safely delivered to mechanically ventilated baboons with ALI, and that COHb levels can be accurately predicted using the CFK equation[38]. We recently completed a fixed dose Phase I trial of iCO in sepsis-induced ARDS (NCT02425579), which showed that precise delivery of low dose iCO is feasible and safe in mechanically ventilated ARDS patients[112]. As anticipated, we found that patients with ARDS of varying severity had different degrees of CO uptake with fixed iCO dosing. We demonstrated that the CFK equation is highly accurate at predicting COHb levels, suggesting that the CFK equation can be used to individually titrate iCO dosing to ensure consistent and safe systemic uptake in ARDS patients with varying degrees of impaired gas exchange.

This Phase Ib safety trial is to evaluate a novel CFK equation-based iCO dosing algorithm to safely and accurately administer low dose iCO in a personalized medicine approach in patients with pneumonia and/or sepsis-induced ARDS that follows our pre-clinical animal studies submitted to the FDA, as well as our published phase 1 safety study. This innovative approach will allow us to determine an optimal and safe dose in real-time for patients with pneumonia, sepsis, and ARDS. Importantly, this study affords a unique opportunity to investigate functional biological signatures underlying the beneficial effects of iCO and their correlation with clinical outcomes in pneumonia, sepsis, and ARDS. We hypothesize that precision-based iCO therapy will be safe, well-tolerated, and reduce the severity of pneumonia and ARDS and organ failure by suppressing mitochondrial dysfunction, inhibiting inflammasome activation and necroptosis, and accelerating resolution of inflammation.

4.3 JUSTIFICATION FOR DOSE

Subjects randomized to CO will be administered algorithm-specified iCO dose (not to exceed 500 ppm) to achieve a COHb of 6-8% for up to 90 minutes for three consecutive days. The decision to target a COHb level of 6-8% is based on animal studies demonstrating protection in sepsis and ALI models **(Table 1)**[24-26,28,30,32,33,38,45-55] and human studies demonstrating safety **(Table 2)**[35,101,104,105,107,108,129] at these COHb levels.

Given diffusion impairment in patients with ARDS, it is anticipated, based on CFK equation predictions **(Table 4)**, that subjects will require doses in the range of 200-500 ppm to achieve COHb levels of 6-8%. The decision to limit the maximal dose to 500 ppm is based on concern for potential epithelial toxicity at inhaled concentrations above 500 ppm. Previous studies have demonstrated safety of CO inhalation at 500 ppm in humans[105] and reduction of pulmonary neutrophilia in non-human primates[54], therefore we do not anticipate epithelial toxicity at our maximal dose.

The rationale for once-daily dosing as opposed to continuous inhalation, as with inhaled NO or other pulmonary vasodilators, is based on pre-clinical studies showing that CO treatment for short periods of time induces transcriptional programs of mitochondrial biogenesis[36,40,41,130] and induces autophagy[33,39,40,131-133] leading to downstream protection without the need for continuous inhalation. In addition, prior work from our group demonstrated safety and activation of mitochondrial biogenesis in healthy individuals after three consecutive days of treatment[10,35]. Once-daily iCO dosing will also optimize subject safety by ensuring that COHb levels do not exceed our target range of 6-8%, and that CO accumulation will not occur as treatments are separated by 4-6 half-lives for CO elimination[134-136].

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), **Section 1.3**. The end of the study is defined as completion of the last visit or procedure shown in the SoA in the trial globally.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

All patients (age 18 and older) will be eligible for inclusion if they meet all of the following consensus criteria for sepsis and ARDS[3,4] or if they meet the criteria for pneumonia as described below.

- Patients with sepsis are defined as those with life-threatening organ dysfunction caused by a dysregulated host response to infection:
    1. Suspected or proven infection: Sites of infection include thorax, urinary tract, abdomen, skin, sinuses, central venous catheters, and central nervous system
    2. Increase in Sequential Organ Failure Assessment (SOFA) Score ≥ 2 over baseline

- ARDS is defined when all four of the following criteria are met:
    1. A PaO_2/FiO_2 ratio ≤ 300 with at least 5 cm H_2O positive end-expiratory airway pressure (PEEP)
    2. Bilateral opacities on frontal chest radiograph (not fully explained by effusions, lobar/lung collapse, or nodules) within 1 week of a known clinical insult or new or worsening respiratory symptoms
    3. A need for positive pressure ventilation by an endotracheal or tracheal tube
    4. Respiratory failure not fully explained by cardiac failure or fluid overload; need objective assessment (e.g., echocardiography) to exclude hydrostatic edema if no risk factor is present

- Pneumonia (without ARDS or sepsis) will be defined as a unilateral or bilateral lung infiltrate on chest X-ray or chest CT (not fully explained by effusions, lobar/lung collapse or nodules) in the setting of receiving mechanical ventilation, a new suspected respiratory infection, an increase in SOFA score less than 2 at the time of randomization (baseline).

- Pneumonia (with sepsis, without ARDS) will be defined as a unilateral or bilateral lung infiltrate on chest X-ray or chest CT (not fully explained by effusions, lobar/lung collapse or nodules) in the setting of receiving mechanical ventilation and a new suspected respiratory infection with an increase in SOFA score of ≥ 2 over baseline at the time of randomization. Pneumonia with bilateral opacities, PaO_2/FiO_2 ratio ≤ 300, or an increase in SOFA score greater than or equal to 2 over baseline will continue to be considered ARDS and sepsis.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Age less than 18 years
2. Greater than 168 hours since ARDS or pneumonia with mechanical ventilation onset
3. Pregnant or breastfeeding
4. Prisoner

5. Patient, surrogate, or physician not committed to full support (exception: a patient will not be excluded if he/she would receive all supportive care except for attempts at resuscitation from cardiac arrest)
6. No consent/inability to obtain consent or appropriate legal representative not available
7. Physician refusal to allow enrollment in the trial
8. Moribund patient not expected to survive 24 hours
9. No arterial line or central line/no intent to place an arterial or central line
10. No intent/unwillingness to follow lung protective ventilation strategy for subjects with ARDS
11. Severe hypoxemia defined as $SpO_2 < 95$ or $PaO_2 < 90$ on $FiO_2 \geq 0.9$
12. Hemoglobin < 7.0 g/dL
13. Subjects who are Jehovah's Witnesses or are otherwise unable or unwilling to receive blood transfusions during hospitalization
14. Acute myocardial infarction (MI) or acute coronary syndrome (ACS) within the last 90 days
15. Coronary artery bypass graft (CABG) surgery within 30 days
16. Angina pectoris or use of nitrates with activities of daily living
17. Severe cardiopulmonary disease classified as New York Heart Association (NYHA) class IV
18. Stroke (ischemic or hemorrhagic) within the prior 1 month, cardiac arrest requiring CPR within the prior 72 hours, or inability to assess mental status following cardiac arrest
19. Burns > 40% total body surface area
20. Severe airway inhalational injury
21. Use of high frequency oscillatory ventilation
22. Use of extracorporeal membrane oxygenation (ECMO)
23. Use of inhaled pulmonary vasodilator therapy (eg. nitric oxide [NO] or prostaglandins)
24. Diffuse alveolar hemorrhage from vasculitis
25. Concurrent participation in other investigational drug study

Reasons for Exclusions:

Patients less than 18 years of age are excluded because the participating ICUs do not typically admit pediatric patients and we believe the benefit/risk ratio for children to receive iCO is not appropriate at this early stage. Patients with ARDS or pneumonia with mechanical ventilation for more than 168 hours are excluded in order to evaluate more clearly the effects of iCO early in the course of lung injury. Patients with severe hypoxemia or need for ECMO are excluded because they may not have adequate reserve to tolerate the reduction in oxygen carrying capacity. The parameters of $SpO_2 < 95$ or $PaO_2 < 90$ on $FiO_2 \geq 0.9$ are used rather than a simpler PaO_2/FiO_2 ratio of 100 to define severe hypoxemia for both enrollment and daily treatment to better account for factors affecting arterial oxygen content and to ensure an additional safety margin during treatment of enrolled subjects. These criteria exclude patients with $PaO_2/FiO_2 \leq 100$ (PaO_2 90/FiO_2 0.9), but also explicitly specify a safety margin of 0.1 for FiO_2. These criteria are currently approved by the Institutional Review Boards (IRBs) at our participating clinical centers, our DSMB, and the FDA for our Phase II CO ARDS trial (NCT03799874).

Patients with hemoglobin < 7.0 g/dL, Jehovah's witnesses, or patients otherwise unable or unwilling to receive blood transfusions during hospitalization are excluded because of the volume of blood drawn for monitoring during iCO therapy may place these patients at greater risks from complications of anemia. Moribund patients and patients with extensive body surface area burns have a high incidence of AEs and lactic acidosis that will confound the safety assessment. Pregnancy, recent stroke, or cardiac arrest are exclusions because iCO may reduce oxygen delivery to the fetus and recently injured brain respectively. Patients with alveolar hemorrhage from vasculitis are excluded because the mechanism of lung injury is

different from ARDS and diffuse alveolar damage. Patients with acute MI within 90 days, recent CABG, and angina pectoris are excluded because of a potential excess risk of reducing oxygen delivery to the myocardium. Patients with NYHA class IV cardiopulmonary disease are excluded because of concerns about ventricular arrhythmias and high mortality. Patients ventilated with high frequency oscillatory ventilation are excluded because administration of iCO with this mode of ventilation may be potentially unreliable. Patients on ECMO are also excluded because the effects of CO uptake and elimination from the blood during ECMO are unknown. Patients on concurrent inhaled pulmonary vasodilator therapy are excluded as these inhaled medications may interfere with the dosing of iCO.
Patients who are cigarette smokers, but otherwise eligible for inclusion, will not be excluded from potential enrollment in the study. Rather, enrolled subjects will be screened with daily COHb levels and study drug treatment held if COHb ≥ 3%. This criterion was implemented for our Phase Ia study and will be used in this trial as smoking history obtained from surrogate decision makers may be unreliable and the average COHb half life is 240 minutes when breathing room air and less on supplemental oxygen[134-136].

Exclusion on the basis of smoking history would preclude many patients from receiving this potentially beneficial treatment. Instead, this protocol incorporates treatment holds on the basis of daily COHb levels, which will allow otherwise eligible patients to be treated safely and have the opportunity to benefit from this potentially new promising therapy. We anticipate enrolling smokers at a frequency similar to their proportion in our target populations.

5.3 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE). Subjects who are screened but fail to be enrolled in the study for any reason will be documented on the screening log along with the reason for screen failure.

Individuals who do not meet the criteria for participation in this trial (screen failure) because of because of severe hypoxemia (Exclusion Criterion 11), low hemoglobin (Exclusion Criterion 12), use of high frequency oscillatory ventilation (Exclusion Criterion 21), or use of inhaled pulmonary vasodilators (Exclusion Criterion 23) may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.4 STRATEGIES FOR RECRUITMENT AND RETENTION

Thirty-six inpatient subjects will be recruited for this study at four enrolling medical centers. Subjects will be identified by prospective medical record review. We anticipate that the distribution of gender, race, and ethnicity of the subjects enrolled will be similar to the demographic characteristics of ICU patients admitted to each of our participating centers and be proportional to the enrollment distribution. No gender, race, and/or ethnicity group will be excluded from participation in our proposed study.

Study coordinators at each site will screen inpatient ICUs daily to identify all potential subjects based on the pre-defined inclusion and exclusion criteria. The recruitment process will consist of electronic medical record (EMR) review of all intubated patients in the ICUs at each site to determine if a potential subject

meets study eligibility criteria. EMR alerts may also be used to assist research coordinators in the early identification of potential participants that meet study eligibility criteria. The research coordinators at each site will identify potential participants and review the study eligibility criteria in detail with the site PI or designee. No recruitment or advertisement materials will be utilized to recruit patients, however flyers may be posted in ICU staff areas to educate colleagues and other health care providers about study eligibility criteria and the importance of participation in clinical research. We will also engage the ICU clinical communities through conferences and other educational resources at each site as our ICU colleagues will play a critical role in the recruitment, retention, and overall conduct of the clinical trial.
Participants will be hospitalized in the ICU at the time of informed consent and throughout the treatment period of the study protocol, thus we do not foresee difficulties in adherence with the study drug treatment. For participants who are extubated prior to completion of the 3-day treatment portion of the protocol, all data will be analyzed on a modified intention-to-treat (MITT) basis. For long-term follow-up outcomes, we will use web-based investigator tools for messaging and scheduling reminders to contact participants.

Several engagement strategies will be adopted to ensure retention of study participants for assessment of long-term outcomes following hospitalization. First, we have designed the study protocol to minimize participant burden in order to optimize subject retention in the trial. We have designed the protocol such that the long-term follow up requires no travel or costs and entails four brief (2-10 minutes) telephone calls at day 28, day 60, 3 months, and 6 months. Second, we have established an efficient tracking system for long-term follow-up. At the time of consent, participants and/or their legally authorized representative (LAR) will be asked to provide email addresses and several phone numbers (home, work, cell) and to indicate the best times to contact them and whether voice and/or text messages can be left.

As many ICU patients are discharged to long-term care facilities following hospitalization, we will consent study participants for permission to contact them at a long-term care facility if applicable. The names and contact information of up to three people who can be contacted for assistance with locating participants will also be requested. Written informed consent to contact these individuals will be obtained, including a description of the scope of the information to be obtained (eg. forwarding addresses versus questions about the clinical status of the participant). In the event of being unable to contact study subjects, we will send letters and/or emails to participants who are unresponsive to personal contact. We will also obtain consent to use social security numbers to determine vital status of participants lost to follow-up.

To mitigate retention risks at the time of consent, we will educate subjects (and their LARs) about their important role as research participants, the requirements of the study, and clearly orient participants to the study demands, tasks, and responsibilities. Most of the patients approached for participation in this research protocol will have limitations of decision-making abilities due to their critical illness or need for sedating medications. Hence, most patients will not be able to provide informed consent and accordingly, informed consent will be sought from the potential subject's LAR. See **Section 10.1.1** for additional information.

We have expanded inclusion criteria to include subjects with unilateral or bilateral infiltrates intubated for suspected lung infection (pneumonia), given similar outcomes from unilateral or bilateral lung infiltrates in critically ill patients, consistency with our preclinical baboon model of ALI due to pneumococcal pneumonia that had improved outcomes with inhaled CO, and the need to expand enrollment for this study.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Inhaled CO or placebo air will be administered to mechanically ventilated subjects using a mechanical ventilator approved for inhaled NO delivery and the CO Delivery System developed by 12th Man Technologies **(Figure 3, Appendix A)**. See **Appendix A** for details of the CO Delivery Device testing, assembly, calibration, and standard operating procedures (SOP). The CO Delivery System will be calibrated and connected to the ventilator **(Figure 9)** as described in **Appendix A**. As per the SOP and illustrated in the schema in **Figure 9**, the injector module will be connected between the inspiration port of the ventilator and inlet port of the humidifier. The gas sampling line will be placed between the outlet port of the humidifier and the patient wye as shown.

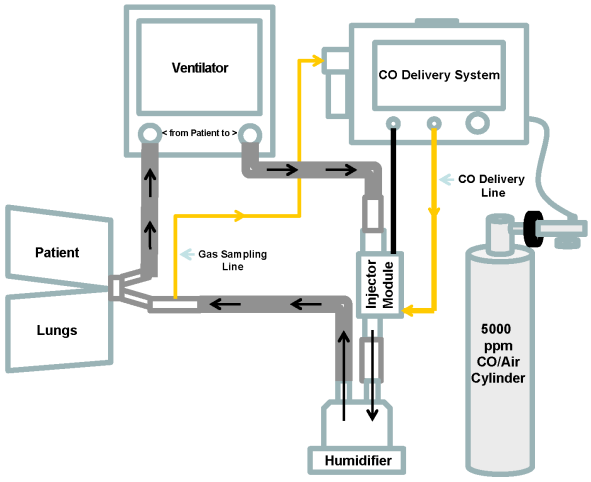


Figure 9. Schema of CO Delivery System and ventilator.

The study drug will be administered by an RT and a physician study staff member OR a physician study staff member alone. A physician study staff member will be present at the bedside during and immediately available for an additional 90 minutes after administration of the study drug to address any clinical concerns that arise, or provide any necessary emergency care in conjunction with the subject's clinical ICU team. The administering RT and physician study staff member OR the physician study staff member alone will be unblinded to the treatment assignments and will conceal the gas cylinders and COventDS to assure that the study coordinator and treating ICU team remain blinded to the study drug assignment.

6.1.2 CO OR PLACEBO CYLINDERS

The gas cylinders for this clinical trial are AG or A3 aluminum cylinders with a CGA 500 valve and will be supplied by Praxair Healthcare Services (Morrisville, PA). The cylinder contains approximately 360 liters of 5000 ppm (0.5%) CO in room air (21% oxygen) and poses no increased flammability risk. Placebo tanks will contain medical grade air in identical appearing cylinders. The gas cylinder's nominal size is 5 inches in diameter by 17 inches tall. Given the flow limitation from the regulator of ~7 liters per minute and a minimum of 6 air exchanges per hour in an average 15x15x10 ICU room, we do not expect ambient CO levels to exceed the OSHA permissible exposure limit (PEL) of 50 ppm as an 8 hour time-weighted average. We measured ambient CO levels during our animal studies using the COventDS and were unable to detect increases in ambient CO levels throughout assembly, calibration, and delivery of CO to the animals. In addition, we simulated CO administration with an ICU ventilator (Puritan-Bennett 840), CO Delivery System, and lung model in an ICU room at Massachusetts General Hospital at 500 ppm for over 2 hours and ambient CO levels were near zero and well below the OSHA PEL.

6.1.3 DOSING AND ADMINISTRATION

Inhaled CO or placebo air will be administered to participants via the COventDS, which will be calibrated and connected to the ventilator **(Figure 9)**. Ventilator management will follow the ARDS Network lower tidal volume (6 mL/kg PBW) protocol[13]. Subjects randomized to CO will be administered iCO at 200 ppm for 20 min as a "test dose" on each treatment day to determine in real-time each subject's CFK equation-determined personalized dose.

COHb levels will be measured before and after the 20 min test dose, and these two levels entered into the CFK equation calculator program to estimate DL_{CO} and compute the iCO dose to achieve a COHb level of 7% (target 6-8%) at 90 min. After the iCO dose is determined, subjects will be administered iCO at the CFK equation-determined personalized dose (200-500 ppm) but **not to exceed 500 ppm or 90 min total (including test dose)**. Subjects randomized to placebo will be administered medical grade air.

The study drug will be administered for up to 90 minutes daily for three days following randomization or until discontinuation of mechanical ventilation, whichever occurs first. For patients who have a tracheostomy, the equivalent of extubation for the purposes of this protocol will be breathing via tracheostomy with unassisted breathing.

The study drug concentration will be monitored continuously with the COventDS built-in analyzer and blood will be drawn at serial time points for arterial blood gas (ABG) analysis and safety monitoring of COHb levels as described in **Section 6.1.4**.

6.1.4 CO MONITORING

The study drug concentration will be monitored continuously with the COventDS built-in analyzer to assure that the accurate dose is delivered. A physician investigator will assess subjects prior to daily iCO treatment. Subjects will have blood drawn daily for measurement of COHb and lactate prior to study drug administration. If COHb ≥ 3% (assessed by unblinded physician) or lactate ≥ 4 mmol/L, the study drug will be held and the subject will be reassessed for treatment the following day. An electrocardiogram (EKG) will also be performed daily prior to study drug administration to evaluate for cardiac exclusion criteria. Blood COHb levels will be measured with an IL GEM Premier Co-oximeter and SpCO measured with a noninvasive pulse oximeter (Masimo Radical-7).

All CO monitoring will be carried out by the administering RT and physician study staff member OR physician study staff member alone and concealed from the study coordinator and clinical team. The physician study staff member will be responsible for maintaining a separate password-protected limited-access project within the database with the subject identification number and CO-related measurements (SpCO, COHb, ambient CO) which the study coordinator will not have access to.

Blood for COHb Monitoring

Blood (arterial or venous) will also be drawn at 20, 40, 60, 75, 90, and 180 minutes for safety monitoring of COHb levels. If an arterial line is not available, venous blood will be used for COHb monitoring as we[38] and others[137-139] have shown that venous levels are as accurate and reliable as arterial measurements. Blood (arterial or venous) will also be drawn for blood gas analysis (using the IL GEM Premier Co-oximeter or local clinical lab) at 90 minutes or at the completion of treatment, if discontinued sooner than 90 minutes. If arterial blood is not available and venous blood gas is used, PaO_2/FiO_2 will be estimated using the formula $SpO_2/FiO_2 = 64 + 0.84 \text{ X } PaO_2/FiO_2$, as long as SpO2 ≤ 97% and subject remains intubated as described in the Statistical Analysis Plan.

The unblinded physician will review and adjudicate COHb measurements and communicate any study drug treatment holds to the administering study staff. COHb levels will be entered into the electronic case report forms (eCRFs) in a separate limited-access project in StudyTRAX by the unblinded RT or physician. The study drug will be administered for up to 90 minutes daily for up to 3 days following randomization or until discontinuation of mechanical ventilation, whichever occurs first.

COVentDS CO Monitoring

The concentration of the study drug will be measured by the built-in gas monitor in the COventDS. The CO Delivery System contains an inhaled gas monitor, which is an electrochemical device that monitors the inhaled gas for concentrations of CO (0-800 ppm) and O_2 (15-100%) to assure that safe levels are inhaled. The sample pump maintains a constant flow of gas to the sensors. Samples of inspired gases are taken with a continuous ~400 mL/min sample pump just proximal to the patient's airway to reflect actual inspired gases. Alarms will sound on high or low CO or O_2 concentrations.

Ambient Air Monitoring

Ambient air CO concentrations will be measured in real time with a Dräger Pac 7000 CO detector to assure that ambient levels are maintained within the recommended limits for occupational exposure of a maximum of 50 ppm. Ambient air CO detectors will be calibrated every 6 months per the manufacturer's instructions to ensure proper functioning.

6.1.5 CO DOSING ALGORITHM USING THE CFK EQUATION

For subjects randomized to iCO, iCO (200 ppm) will be administered for up to 20 minutes as a "test dose" on each day to determine the subject's CFK equation-determined dose. The COHb level will be measured prior to the test dose and 20 minutes after the test dose. These two COHb levels will be used in the CFK equation to determine each subject's specified dose in order to achieve a COHb level of 6-8%. After the CO dose is determined, subjects will be administered inhaled CO at the algorithm-determined dose- not to exceed 500 ppm or 90 minutes total (including test dose). Subjects randomized to placebo will be administered medical grade air.

1. Arterial or venous blood will be drawn for baseline blood gas analysis and COHb measurements prior to iCO or placebo administration.
2. Subjects randomized to CO will be treated with inhaled CO at 200 ppm for 20 minutes (test dose).
3. After 20 minutes, blood will be drawn for COHb measurement.
4. DLCO will be estimated using the baseline and 20 min COHb in the CFK equation calculator program, and used to compute the iCO dose to achieve a COHb level of 7% (target 6-8%) at 90 minutes.
5. Dose will be adjusted (200-500 ppm) in subjects randomized to CO based on CFK equation-determined dose.
6. Blood will be drawn for COHb measurements at 20 min, 40 min, 60 min, 75 min, 90 min, and 3 hours. Blood will also be drawn for blood gas analysis at 90 min.

On days 1-3, a total of approximately 3 mL of blood will be drawn during the study drug administration for safety monitoring of COHb levels and blood gas analysis.

6.1.6 DAILY HOLD PARAMETERS PRIOR TO STUDY DRUG ADMINISTRATION

Subjects will be assessed daily prior to study drug administration as described in the **Schedule of Activities (Section 1.3)**.

Administration of the study drug will be held if the following criteria are met.

- COHb level ≥ 3%
- Lactate ≥ 4 mmol/L
- ST elevation MI or unstable angina/non ST elevation MI concerning for ACS
- Unstable atrial or ventricular arrhythmia

Subjects will have blood drawn daily for measurement of COHb and lactate prior to administration of study drug. If COHb ≥ 3% or lactate ≥ 4 mmol/L, the study drug will be held until the next scheduled dose the following day. Lactate and COHb will be measured the following day to determine whether the study drug will be administered. If the study drug is being held for another reason, COHb levels will not be measured on days the study drug is being held.
A 12 lead EKG will also be performed and reviewed daily by the study staff physician prior to study drug administration to evaluate for cardiac exclusion criteria (eg. acute MI, acute coronary syndrome (ACS), or unstable atrial or ventricular arrhythmia). If a subject meets the following criteria for ST elevation MI or unstable angina/non ST elevation MI concerning for ACS according to the American College of Cardiology Foundation/American Heart Association guidelines[140-142], they will be excluded from enrollment or further study drug administration according to exclusion criteria.

ST elevation MI (STEMI) Criteria: New ST elevation at the J point in at least 2 contiguous leads of ≥ 2 mm (0.2 mV) in men or ≥ 1.5 mm (0.15 mV) in women in leads V2–V3 and/or of ≥ 1 mm (0.1 mV) in other contiguous chest leads or the limb leads; New or presumably new left bundle branch block (LBBB)[140-142].

Unstable angina/non ST elevation MI (NSTEMI): Ischemic ST-segment depression ≥ 0.5 mm (0.05 mV) or dynamic T-wave inversion with pain or discomfort; Nonpersistent or transient ST-segment elevation ≥ 0.5 mm for < 20 minutes. Threshold values for ST-segment depression consistent with ischemia are J-point depression 0.05 mV (-0.5 mm) in leads V2 and V3 and -0.1 mV (-1 mm) in all other leads (men and women)[140-142].

Note, troponin (I or T) may be increased in patients with sepsis and ARDS[143] in the absence of an acute MI or ACS from coronary artery disease. If, in the judgment of the clinical team, an ARDS patient with elevated troponin levels has no other indication of an MI or ACS, the patient may still be eligible for enrollment.

In addition, if a subject develops the following criteria during the study, the study drug will be held until resolved:

- Severe hypoxemia defined as $SpO_2 < 95$ or $PaO_2 < 90$ on $FiO_2 \geq 0.9$
- Hemoglobin < 7.0 g/dL
- Diffuse alveolar hemorrhage from vasculitis
- Use of high frequency ventilation
- Use of inhaled pulmonary vasodilator therapy (eg. NO or prostaglandins)
- Use of ECMO

If study drug hold criteria are met, the study drug will be held and subjects will be evaluated on a daily basis and assessed for whether a given criteria has resolved and whether study drug treatment may be resumed.

6.1.7 INTERRUPTION OF DOSING DURING STUDY DRUG ADMINISTRATION

Subjects will have blood drawn for measurement of COHb levels prior to study drug treatment and 20 min, 40 min, 60 min, 75 min, 90 min, and 180 min after the start of study drug treatment. It is anticipated that COHb levels will not exceed the target range of 6-8% given the impaired diffusion capacity in ARDS patients and CFK equation-determined personalized iCO dosing algorithm. However, the following parameters will be used to shorten the 90 minute treatment duration should CO uptake be higher than anticipated:

The study drug will be stopped prior to 90 minutes:

1. If the measured COHb level is > 8% at any time during study drug treatment.
2. If the investigator, attending physician, the patient, or their surrogate decides that the study drug should be discontinued.

If COHb levels unexpectedly exceed our target level, in addition to discontinuation of the study drug, subjects will be placed on 100% O_2 to accelerate CO elimination until COHb levels normalize.

6.1.8 PERMANENT DISCONTINUATION OF STUDY DRUG

Permanent discontinuation of the study drug is defined as cessation of the study drug without the intent of restarting the study drug during the three-day treatment period.

Permanent discontinuation of the study drug inhalation will occur in the following situations:

- **Occurrence of pre-specified administration-related AEs:**
  - Acute MI* within 48 hours of study drug administration
  - Acute stroke** within 48 hours of study drug administration
  - New onset atrial or ventricular arrhythmia requiring DC cardioversion within 48 hours of study drug administration
  - Increased oxygenation requirements defined as: an increase in FiO_2 of ≥ 0.2 AND increase in PEEP ≥ 5 cm H_2O within 6 hours of study drug administration.
  - Increase in any protocol-specified measurement of COHb ≥ 10%
  - Increase in lactate by ≥ 2 mmol/L within 6 hours of study drug administration
- If the subject experiences serious adverse events (SAEs) **related to the study drug**
- If the investigator, attending physician, the patient or their surrogate decides that the study drug should be discontinued. If this decision is made because of an AE, then appropriate AE reporting procedures will be followed **(Section 8.7)**.
- Daily baseline COHb levels ≥ 3% leading to three missed drug doses
- Three or more missed drug doses due to AEs

* Acute MI is defined below according to the Executive Group on behalf of the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC)/American Heart Association (AHA)/World Heart Federation (WHF) Task Force for the Universal Definition of Myocardial Infarction[144]. We will also rely upon subspecialty consultation to confirm the presence or absence of an acute MI.

Acute myocardial infarction is defined as acute myocardial injury with clinical evidence of acute myocardial ischemia and with detection of a rise and/or fall of cardiac troponin values with at least 1 value above the 99th percentile upper reference limit and at least 1 of the following:

- Symptoms of myocardial ischemia;
- New ischemic ECG changes;
- Development of pathological Q waves;
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischemic etiology;
- Identification of a coronary thrombus by angiography or autopsy (not for types 2 or 3 MIs).

** Acute stroke is defined as an acute neurological deficit attributed to an acute focal injury of the central nervous system by a vascular cause, including cerebral infarction, intracerebral hemorrhage, and subarachnoid hemorrhage based on neuropathological, neuroimaging, and/or other clinical evidence of permanent injury[145]. We will also rely upon subspecialty consultation to confirm the presence or absence of an acute stroke.

Subjects who have their study drug permanently discontinued will continue their participation in the study, and will be followed to determine their vital status and perform neurocognitive testing at 3 and 6 months, as outlined in the **Schedule of Activities (Section 1.3)**.

6.1.9 COMPLETION OF THE STUDY DRUG ADMINISTRATION

Subjects will be considered to have completed the study drug administration portion of the study when one of the following conditions is met, whichever comes first:

1. Three days after study drug administration
2. Discontinuation of mechanical ventilation
3. Death
4. Pre-specified criteria met for permanent discontinuation of study drug **(Section 6.1.8 in the Study Protocol)**

6.1.10 TREATMENT IN COVID-19 SUBJECTS

For enrolled pneumonia or ARDS patients with COVID-19 infection, all research and clinical staff will conform to each site's hospital infection control guidelines for COVID-19 positive patients with appropriate personal protective equipment (PPE) for enhanced respiratory isolation precautions. All research and clinical staff will have appropriate training on PPE, be fit-tested, and wear an N95 respirator or powered air-purifying respirator (PAPR) if staff cannot be fit-tested, face shield or other eye protection, gloves, and gown when entering the subject's room for treatment or sample collection. Gown, gloves, and eye protection will be doffed upon exiting the subject's room either just inside or just outside the door, and hand hygiene will be performed. Respiratory protection should be removed after exiting the subject's room unless extended use is being practiced.

During study drug treatment, all staff will conform to each site's hospital infection control guidelines regarding treatment of COVID-19 positive mechanically ventilated patients with inhaled therapies. Staff will be limited to essential personnel in the room. All equipment will be single-use or dedicated to use of

the subject to avoid sharing with other subjects. Reusable equipment will be disinfected with a hospital-approved disinfectant before use in another subject.

Blood sample collection and handling will be performed according to each site's hospital infection control guidelines for COVID-19. Samples obtained from a subject will be put in a tube by research or clinical staff, and the tube will be wiped down with a hospital-approved disinfectant. While holding the tube with the wipe, the specimen tube will be carefully placed into a clean specimen bag held by a monitor or nurse outside the room. The specimen bag will in turn be wiped down with a hospital-approved disinfectant. A member of the research team will pick up the research specimens and transport them to a BSL2+ laboratory in a secondary container. Research samples will only be processed in hospital biosafety committee approved BSL2+ laboratory facilities.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Each participating site is responsible for the ordering, storing and dispensing of the investigational agent(s). Each study team will be responsible for keeping a Site Drug Accountability Log at the site which will include a log of CO/placebo air tanks with lot number/cylinder number, concentration, expiration date, and dates received from/returned to Praxair. A subject study drug accountability log will record the subject ID, cylinder number, content of tank, dose (ppm), date dispensed, and tank pressure at start/end of treatment.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The study drug will be provided for this study by Praxair Healthcare Services. Gas mixtures will be manufactured with United States Pharmacopeia (USP)/Ultra High Purity (UHP)/National Formulary (NF) components. The mixtures will be prepared as a Certified Standard with a blend tolerance of ± 10% and analytical uncertainty of 2%.

The gas mixture provided to subjects randomized to CO will be 5000 ppm CO (balance air) and will be provided to study sites by Praxair Healthcare Services in AG or A3 aluminum cylinders with a CGA 500 valve. The gas cylinder's nominal size is 5 inches in diameter by 17 inches tall, contains 360 liters of 5000 ppm (0.5%) CO in room air (21% oxygen), and poses no increased flammability risk. The 5000 ppm CO source gas will be administered via the CO Delivery System (12th Man Technologies, Garden Grove, CA) and delivered to subjects at a CFK equation-determined individualized dose (200-500 ppm) to achieve a target COHb level of 6-8%.

The mixture components include: **Carbon Monoxide UHP Grade 3.0** (Assay 99.9%; O_2 <10 ppm, H_20 <5 ppm, N_2 <900 ppm and CO_2 <20 ppm); and **Medical Air USP**. The source materials for **Medical Air USP** include: **Oxygen USP** (Assay ≥ 99.2%; Odor- none; Oxygen produced by air liquefaction process, does not require CO and CO_2 analysis); and **Nitrogen NF USP** (Assay ≥ 99.2%; Identification- OK, Paramagnetic Assay Method; CO ≤ 10 ppm; Odor- none).

The gas mixture provided to subjects randomized to placebo will be medical grade air and will be provided to study sites by Praxair Healthcare Services in AG or A3 aluminum cylinders with a CGA 346 valve. With the exception of the 346 valve, the placebo gas cylinders will be identical in size and color as the CO cylinders. The placebo gas will be administered via the CO Delivery System (12th Man Technologies,

Garden Grove, CA) and delivered to subjects in an identical fashion as iCO delivery.

6.2.3 PRODUCT STORAGE AND STABILITY

The CO and placebo air cylinders will be stored at room temperature in a secure area at each center. The cylinders will be stored and handled according to each site's Compressed Gas Cylinder Storage Guidelines. The CO and placebo air cylinders will be returned to Praxair upon expiration. The expiration date is generally three years from the date of fill and is clearly indicated on the certificate of analysis for each cylinder.

6.2.4 PREPARATION

As described in the SOP, the CO Delivery System will be calibrated and performance testing conducted daily prior to use. The calibration will be performed as described in the Operator's Manual in **Appendix A**. The calibration consists of a low (21%) and high (100%) range O_2 calibration, followed by a low (100 ppm) and high (400 ppm) range CO calibration. After completion of the calibration, performance testing will be performed as described in the SOP. The CO Delivery System will be assembled, the appropriate gas cylinder (CO or air) connected, and the delivery system will be connected to the ventilator as described in **Appendix A**. As shown in **Figure 9**, the injector module will be connected between the inspiration port of the ventilator and inlet port of the humidifier. The gas sampling line will be placed between the outlet port of the humidifier and the patient wye as shown.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Eligible participants will be randomized to one of the two treatment arms using a permuted block method[146] with random block sizes. The randomization ratio will be 2:1 iCO:placebo. The randomization will not be stratified by any factors. The study biostatistician will verify all the necessary randomization information (blinding method, randomization method, block size, and number of treatment groups) in the StudyTRAX randomization configuration module.

Site investigators will review all potential study participants with one of the CCC physician members. Following informed consent, the unblinded administering RT or physician or blinded study coordinator will randomize subjects in the database using the StudyTRAX randomization tool. The unblinded administering RT or physician will obtain the subject's treatment assignment through the "View Treatment" button in the blinded database, a privilege granted to unblinded RTs and physicians only. CO-related measurements (SpCO, COHb, ambient CO) and drug-related information will be in a separate project within the database, which the blinded study coordinators will not have access to. Permission to this limited-access project will only be granted to unblinded study personnel by the PI, as it contains treatment-specific information such as CO monitoring and study drug administration data.

The study drug assignment will be blinded to the subject, clinical team, study coordinators, and other study staff with the exception of the administering study staff (RT and physician OR physician alone), who will be unblinded to the treatment assignment to ensure subject safety. We chose this partially double-blind design to ensure safe iCO administration and an unblinded assessment of COHb levels in order not to exceed potentially toxic thresholds.

To maintain blinding, the placebo air cylinders are identical in appearance as the CO cylinders (with the exception of different CGA valves required by the manufacturer Praxair). Cylinders will also be shrouded in dark sleeves to ensure blinding. The placebo air will be administered via inhalation via the COventDS

and delivered to subjects in an identical fashion as those randomized to CO. To ensure that the subject, study coordinator, and clinical staff remain blinded to the study drug assignment, the administering RT and physician study staff member OR physician study staff member alone will conceal the gas cylinders, CO delivery device, and measurements of COHb, SpCO, and ambient CO levels. Unblinded test results will be kept in a separate location from the blinded test results and concealed from the study coordinators.

The unblinded administering physician will be responsible for maintaining a separate password-protected limited-access project within the database with the subject identification number and CO-related measurements (SpCO, COHb, ambient CO) which the study coordinator will not have access to. While the administering investigator will be unblinded throughout the study due to safety monitoring, they will only be unblinded to the treatment assignment for subjects enrolled at their own site. Investigators will otherwise be blinded to the study treatment assignment for subjects enrolled at the other sites. The lead RT (Mr. Davies) may become unblinded to the treatment assignments at other sites to assist with study drug administration procedures if necessary.

If the clinical team believes that unblinding is medically necessary in order to properly treat the patient, the unblinded administering physician can disclose study product assignment. If there is time to do so, the unblinded administering physician should discuss this unblinding with the unblinded CCC physician prior to the disclosure. All episodes of unblinding, whether deliberate or inadvertent, will be reported to the DSMB at the next routine report.

A blinded study physician at each site will review all AEs and will assess their relationship to the study intervention. Additional AE adjudication will be provided by one of the Medical Monitors (Dr. North or Dr. Thompson). In addition to blinded adjudication of AEs, investigators blinded to treatment allocation will assess chest X-rays required for the calculation of the LIS score. De-identified chest X rays at each site will be reviewed centrally by blinded investigators at BWH.

6.4 STUDY INTERVENTION COMPLIANCE

To assess protocol adherence during monitoring visits, the CCC will review the Study Drug Administration and CO Administration Monitoring forms, in addition to the Study Drug Accountability Logs. The administering RT and physician study staff member OR physician study staff member alone will keep track of study drug assignment and dosages, time and duration of treatment, tank lot number, and COHb results on Subject Drug Administration and CO Administration Monitoring forms. These forms should be kept in a secure place and confidential from blinded study staff.

Each participating site is responsible for the ordering, storing and dispensing of the investigational agent(s). Each study team will be responsible for keeping a Site Drug Accountability Log at the site which will include a log of CO/placebo air tanks with lot number/cylinder number, concentration, expiration date, and dates received from/returned to Praxair. A subject study drug accountability log will record the subject ID, cylinder number, content of tank, dose (ppm), date dispensed, and tank pressure at start/end of treatment.

6.5 CONCOMITANT THERAPY

Subjects will be excluded from participation in the study if they are receiving concomitant inhaled pulmonary vasodilators continuously (such as inhaled nitric oxide [NO] or prostacyclin), as these inhaled medications may interfere with the dosing of inhaled CO. If a decision is made by the clinical team to

initiate these medications in enrolled subjects following randomization and within the 3-day study treatment period, the study drug will be held until subjects are no longer receiving these medications concomitantly. The study drug may be reinitiated following cessation of these inhaled medications if there are no other protocol-specified reasons that preclude ongoing treatment in the study. Potential subjects will also be excluded if they are participating in another investigational drug study. There are otherwise no contraindications to concomitant medications in the study.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from study drug does not mean discontinuation from the study, and remaining study procedures should be completed as indicated in the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the site investigator or designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an AE.

Permanent discontinuation of the study drug is defined as cessation of the study drug without the intent of restarting the study drug during the three-day treatment period. Subjects who have their study drug permanently discontinued will continue their participation in the study, and will be followed to determine their vital status and perform neurocognitive testing at 3 and 6 months as outlined in the **Schedule of Activities (Section 1.3)**.

Criteria for permanent discontinuation of the study drug are described in **Section 6.1.8**. In some instances, the study drug may be held and the subject may be re-evaluated for treatment on subsequent days. The criteria for a study drug hold are described in **Section 6.1.6**.

If study drug hold criteria are met, the study drug will be held and subjects will be evaluated on a daily basis and assessed for whether a given criteria has resolved and whether study drug treatment may be resumed.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request. An investigator may discontinue or withdraw a participant from the study for the following reasons:

- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant unable to receive at least one dose of the study drug

If a patient or surrogate requests withdrawal from the study, the investigators will seek explicit permission to continue data collection.

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive at least one dose of the study product, and subsequently withdraw, or are withdrawn or discontinued from the study will not be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if the study staff if unable to verify vital status after the subject has been discharged from the hospital. Before a participant is deemed lost to follow-up, the site investigator or designee will make every effort to regain contact with the participant or surrogate (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

We will verify duration of survival for participants lost to follow-up or noted to have died using the Centers for Disease Control and Prevention's National Death Index (National Death Index, 2000). The subject's social security number (SSN) will be used for an exact NDI match. Contact information will be collected for the subject and alternative contact information will be collected on up to 3 individuals. Contact information and the subject's SSN will be collected on paper at the time of consent. Contact information and SSN will be maintained on paper locally and will not appear in the study database.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 DESCRIPTION OF STUDY PROCEDURES

All clinical study procedures will be performed in accordance with Good Clinical Practice (GCP) standards.

Study assessments described below will be done according to the **Schedule of Activities** in **Section 1.3**.

APACHE II Score: The APACHE II consists of 12 physiological variables, age, and underlying health. The score will be calculated based on the worst values in the 24 hours prior to randomization. Calculation variables are described in **Appendix B1**.

Blood gas analysis: The following values will be recorded from the arterial blood gas (ABG) results: pH, PCO_2, PO_2, and base excess or deficit. ABG is required for baseline assessment. If no ABG is available on subsequent days, record blood gas analysis results from the venous blood gas (VBG).

Calibration of IL GEM Premier Co-oximeter and COventDS: Calibration will be performed according to device manuals and SOP. Documentation of calibration will be kept in study files.

Central Venous Pressure (CVP): CVP will be recorded if available.

Calculate Deadspace (Vd/Vt): Dead space fraction will be measured daily by the administering RT or physician prior to study drug administration using a NICO or NM3 monitor (Respironics) and the PCO_2 obtained from an arterial or venous blood gas.

Chest X-ray (CXR): The baseline frontal CXR will be reviewed by two study physician investigators to determine study eligibility. Study physician investigators blinded to treatment allocation will review and perform the assessment for the Lung Injury Score (LIS). All 4 lung quadrants will be assessed for infiltrates and pneumothoraces. If the subject is extubated on days 1-5, the study team will order a chest X-ray the

day following extubation, if not ordered by the clinical team. Chest X-rays will also be ordered by the study team for endpoint analysis on Day 7, if not ordered by the clinical team.

Clinical Laboratory Assessments: Clinical labs will be processed at each site's local laboratory and reviewed by a study investigator. If labs are required according to the **Schedule of Activities (Section 1.3)** and are not available as part of standard of care testing, they will be drawn for research. A urine or serum pregnancy test will be done for women of childbearing potential. A subject with a positive pregnancy result will not be eligible for randomization.

Concomitant Medications Review: The following medications will be recorded in the study eCRFs: aspirin, COX-2 inhibitors, NSAIDs, ACE inhibitors, steroids, phosphodiesterase inhibitors, neuromuscular blockade, inhaled NO or prostaglandins, antibiotics, diuretics. TPN use will also be recorded.

EKG: A 12 lead EKG will be obtained and reviewed daily by the study staff physician prior to study drug administration to evaluate for cardiac exclusion criteria (acute MI, ACS, or unstable atrial or ventricular arrhythmia). The study drug will not be started until the EKG is obtained and reviewed by the study physician investigator for a potential study hold. The EKG will also be officially read locally at each site.

Fluid Intake/Output: Fluid intake and output (most recent 24 hour value) or mean hourly value for most recently available period. Diuretic administration will also be recorded if applicable.

Glasgow Coma Scale (GCS): The GCS is a determination of the best eye opening, motor, and verbal responses at any given time. The GCS is documented by the clinical team as part of routine ICU nursing assessment. If not available, the GCS will be assessed by the study staff as described in **Appendix B2**.

Lung Injury Score (LIS): The lung injury score will be calculated based on the chest x-ray assessment, PaO_2, FiO_2, PEEP, and ventilator compliance measurement as described in **Appendix B3**.

Microbiological Results: Microbiological results will be recorded in the eCRF when ordered for clinical purposes.

a) Blood cultures
b) Urine cultures
c) Sputum cultures
d) BAL cultures
e) CSF cultures if available
f) Stool
g) Other

Neurocognitive Testing: Neurocognitive testing will be performed via telephone interviews using the Montreal Cognitive Assessment (MoCA)-BLIND and Hayling sentence completion test as **(Appendix C)**.

Oxygenation Index (OI): The OI will be calculated based on the PaO_2, FiO_2, and mean airway pressure obtained from the ventilator parameters.

Renal replacement therapy status: Renal replacement therapy status within the last 24 hours will be recorded.

Richmond Agitation Scale (RASS): RASS is a scale used to measure the agitation or sedation level of a patient. RASS scoring is documented by the clinical team as part of routine ICU nursing assessment. If not available, the RASS will be assessed by the study staff as described in **Appendix B4**.

ScvO2: ScvO2 will be recorded if available.

Sequential Organ Failure Assessment (SOFA): The SOFA score will be calculated based on the worst values recorded in the past 24 hours. Calculation variables are described in **Appendix B5**.

a) Worst PaO_2/FiO_2 for that date
b) Worst creatinine (or urine output), bilirubin, and platelet count for that date
c) Worst Glasgow Coma Scale for that date
d) Vasopressor use and maximal dose for that date

Vasopressors and Inotropes Review: The following vasopressors/inotropes will be recorded in the study eCRFs (epinephrine, norepinephrine, phenylephrine, vasopressin, dopamine, dobutamine, phosphodiesterase inhibitors, including dose).

Ventilator Parameters: The study staff will record ventilator parameters prior to starting study drug administration. The following settings will be recorded:

a) Mode of ventilation
b) FiO_2 and PEEP
c) Tidal volume, Respiratory rate (set rate and total rate), Minute ventilation, Inspiratory flow rate
d) Peak pressure, Plateau pressure, Compliance, and Mean airway pressure. If on a pressure-cycling mode, peak pressure during inspiration will be assumed to be the plateau pressure.
e) Pressure during inspiration if on a pressure targeted mode (PSV, PCV, etc)

Vital Signs: Heart rate (beats/min), systemic systolic, diastolic, mean arterial blood pressure (mm Hg), and body temperature (°C), will be recorded in the eCRF.

Vital Status: Vital status will be assessed at Days 28, 60, 3 months, and 6 months. Subjects who have been discharged from the hospital will be contacted by phone. Additional information will be collected on discharge status, ventilator status, and vasopressor status at Days 28 and 60.

8.1.1 STUDY SAMPLE COLLECTION

Sample collection will be done according to the **Schedule of Activities (SoA)** in **Section 1.3**.

COHb Levels: COHb levels will be measured using the IL GEM Premier Co-oximeter at specified time points on Days 1 through 3. See **Section 8.5.3**.

Plasma Samples: Blood (4 mL) will be collected pre-treatment and post-treatment (90 mins and 3 hrs) on days 1-3 in EDTA anti-coagulated tubes for plasma isolation and measurement of biomarkers of mitochondrial dysfunction, inflammasome activation, lipid mediators, and cytokines. Blood (4 mL) will also be collected for plasma isolation on Day 5. Plasma will be obtained and divided immediately after centrifugation into equal aliquots in specified tubes and frozen at –80°C.

RNA Samples: Blood (2.5 mL) will be collected pre-treatment and post-treatment (90 min and 3 hrs) on days 1-3 in Paxgene tubes for RNA isolation, and frozen at –80°C. Blood (2.5 mL) will also be collected in Paxgene tubes for RNA isolation on Day 5 and frozen at –80°C.

DNA Samples: Cell pellets will be obtained for future DNA isolation from the blood collected in EDTA tubes on days 1-3 and on day 5. Cell pellets will be isolated pre-treatment and post-treatment (at 90 min and 3 hrs) on days 1-3.

Primary Cell Isolation: An additional 8 mL of blood will be drawn for isolation of peripheral blood mononuclear cells (PBMCs) pre-treatment on day 1 and post-treatment on day 3. This tube may be used to isolate cell pellets (for future DNA isolation) if unable to obtain an adequate pellet from the 4 mL tube on days 1 and 5.

Urine Sample Collection: Between 50-100 mL of urine will be collected in a sterile specimen container on days 1-3 and on Day 5. Cell pellets will be isolated pre-treatment and post-treatment (at 90 min and 3 hours) to obtain RNA and protein, as well as mtDNA.

Discarded Specimens: Discarded bronchoalveolar lavage (BAL) fluid and plasma will be obtained (when available) for levels of cytokines, mediators/biomarkers, and protein. Plasma and BAL fluid will be divided into equal aliquots in specified tubes and frozen at –80°C.

A total of approximately 90 mL of blood will be collected for research samples during the study from each study subject as follows:

Sample collection	**Day 1**	**Day 2**	**Day 3**	**Day 5**	**Total Volume**
ABG, lactate, and COHb analysis	3 mL	3 mL	3 mL		**9 mL**
4 mL EDTA tube (plasma and DNA)- pre and post-treatment	12 mL	12 mL	12 mL	4 mL	**40 mL**
2.5 mL PAXgene tube (RNA)- pre and post-treatment	7.5 mL	7.5 mL	7.5 mL	2.5 mL	**25 mL**
Primary Cell Isolation	8 mL			8 mL	**16 mL**
Total Volume	**30.5 mL**	**22.5 mL**	**22.5 mL**	**14.5 mL**	**90 mL**

Additional blood (29 mL) may be drawn during the study for research purposes as below if not drawn as part of usual clinical care.

Sample collection	**Baseline**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 7**	**Day 14**	**Day 28**	**Total Volume**
ABG, lactate	2 mL									2 mL
CBC	2 mL	2 mL	2 mL	2 mL						8 mL
Metabolic panel	3 mL									3 mL
Bilirubin		2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	16 mL
Total Volume	**7 mL**	**4 mL**	**4 mL**	**4 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**29 mL**

Samples will be sent to the central repository at BWH to be stored. Study subject ID numbers will identify samples during shipment and storage in the central repository. In the future, the CCC will instruct the repository to prepare the appropriate samples for shipment to sites for biomarker analyses. The CCC will not record or store unique patient identifiers (such as initials, date of birth, hospital record numbers, addresses, phone numbers, etc.) in the database. All data released by the CCC for studies will be linked to the specimen but will be de-identified. The link (key) between the de-identified database and the subject will be removed two years after the primary publication. Samples collected for this trial will be frozen and

stored in the biorepository for future research related to carbon monoxide, ARDS, sepsis, critical illness or other lung diseases.

8.2 SCREENING PROCEDURES

No procedures will be required to determine subject eligibility. Patients will be screened based on laboratory values (eg. arterial blood gas analysis) and radiologic tests (eg. CXR) that are otherwise conducted as part of routine clinical care in the ICU. No study procedures will be initiated prior to obtaining written informed consent.

During the screening evaluation, the subject's medical history, vital signs, ventilator settings/mechanics, laboratory values, and chest X-ray will be reviewed to determine if the patient meets eligibility criteria to participate in the study.

- Review medical history, vital signs, and concomitant medications
- Review ventilator settings/mechanics and PaO_2/FiO_2
- Review chest X-ray performed as part of clinical care
- Review laboratory values drawn as part of clinical care including arterial blood gas (ABG), lactate, and troponin if available
- Review electrocardiogram (EKG) performed as part of clinical care
- Review urine or blood pregnancy testing if subject is female and able to become pregnant

A screening log will be kept to track subjects that could potentially meet inclusion criteria. All patients meeting the inclusion criteria will be entered into the StudyTRAX database. If the patient is not enrolled, information explaining why enrollment did not occur will be entered into StudyTRAX (exclusion criteria, attending physician denial, patient refusal, etc. see **Section 8.2.2** for a listing of the de-identified data to be collected on screened, non-enrolled subjects).

If any patient meets criteria for study enrollment, the attending physician will be asked for permission to approach the patient or his/her surrogate for informed consent.

8.2.1 ASSIGNING A SCREENING ID

All subjects who are screened will be given a Reference ID and a Project ID. The Reference ID is automatically generated as a consecutive number by StudyTRAX (eg. Ref-0001, Ref-0002). The Reference ID carries with the subject throughout projects; it is universal to the database and does not contain any project-specific information.

Additionally, a specific Project ID will be assigned to each subject that contains the site and a sequence unique to each site as follows (eg. BWH-1b-0001, MGH-1b-0001, BWH-1b-0002 ... WCM-1b-0001). Sites will be identified with a three-letter code as follows: BWH (Brigham and Women's Hospital), MGH (Massachusetts General Hospital), Duke University Hospital (DUH), and Weill Cornell Medicine (WCM). The sites operate as separate projects, therefore coordinators and physicians only have access to the subjects at their site, unless additional privileges/permissions are granted in the database. All subjects who have been screened will be included in the Subject Screening Log.

8.2.2 SCREENED, NOT ENROLLED

Subjects who are screened, but not enrolled into the study, either because they meet one or more exclusion criteria or decline participation, will be assigned a screening ID. The following de-identified data will be collected for all subjects that have been screened, but not consented/enrolled.

- Date of intubation, qualifying PaO_2/ FiO_2 and ARDS onset
- PaO_2
- SpO_2
- FiO_2
- Month of the year that patient met screening criteria (1-12)
- Gender
- Ethnicity
- Age (if age >89, 89 will be entered for age)
- Patient location (e.g. MICU, SICU, etc.)
- Reason(s) patient excluded from study
- If not excluded, not enrolled, why?
- Lung injury category (e.g. sepsis, pneumonia)
- Site of infection if lung injury category is sepsis

This data will be entered into the StudyTrax database under the screening ID.

8.3 RANDOMIZATION PROCEDURES (DAY 0)

8.3.1 RANDOMIZATION

All ARDS criteria (for those enrolled with sepsis-induced ARDS) and pneumonia criteria in ventilated patients must occur within the same 24 hour period. The onset of ARDS is when the last criterion is met. Patients must be enrolled within 168 hours of ARDS onset or diagnosis of pneumonia in a ventilated patient. Information for determining when these time window criteria were met may come from either the site hospital or reports from a referring hospital.

Following randomization, the low tidal volume protocol for mechanical ventilation must be initiated within two hours for those subjects with ARDS..

The first treatment of study drug should be given within 24 hours of randomization. The day of randomization will be considered study day zero.

Eligible participants will be randomized to one of the two treatment arms using a permuted block method[146] with random block sizes. The randomization ratio will be 2:1 iCO:placebo. The randomization will not be stratified by any factors. The study biostatistician will verify all the necessary randomization information (blinding method, randomization method, block size, and number of treatment groups) in the StudyTRAX randomization configuration module.

Site investigators will review all potential study participants with one of the CCC physician members. Following informed consent, the unblinded administering RT or physician or blinded study coordinator will randomize subjects in the database using the StudyTRAX randomization tool. The unblinded administering RT or physician will obtain the subject's treatment assignment through the "View Treatment" button in the blinded database, a privilege granted to unblinded RTs and physicians only. CO-

related measurements (SpCO, COHb, ambient CO) and drug-related information will be in a separate project within the database, which the blinded study coordinators will not have access to. This central review of randomization by the CCC unblinded physician will ensure that no subjects are enrolled in this multi-center trial during planned or unplanned study holds.

8.3.2 ASSIGNING A RANDOMIZATION

All subjects will be randomized through the StudyTRAX database using a permuted block method with random block sizes. The unblinded physicians or RTs or blinded coordinators will randomize the subject after enrollment. Subjects will receive a Randomization ID (RI-SiteCode-Seq) which includes a Site Code and Sequence starting with 0001 per site (eg. RI-BWH-0001, RI-MGH-0001, RI-DUH-0001, RI-WCM-0001).

8.3.3 VENTILATOR PROCEDURES

FiO_2 will be increased prior to study drug administration in order to achieve a $PaO_2 \geq 80$ or $SpO_2 \geq 95\%$. Ventilator management, including weaning, should follow the modified ARDS Network lower tidal volume (6 mL/kg PBW) protocol **(Appendix D)**[13]. Study drug administration will be continued in patients undergoing weaning from mechanical ventilation as in unless the subject is deemed ready for extubation by the clinical team.

8.4 BASELINE ASSESSMENTS

The following baseline previous medical history and admission information will be recorded after informed consent has been obtained and within the 24-hour interval preceding initiation of study drug treatment.

1. Demographic and Admission Data
2. Pertinent Medical History and Physical Examination
3. Height; gender, measured body weight (MBW); calculated predicted body weight (PBW).
4. Time on ventilator prior to enrollment
5. Type of Admission
    a. Medical
    b. Surgical scheduled
    c. Surgical unscheduled
    d. Trauma
6. Presumed site of infection
7. Acute or Chronic renal failure and use of dialysis
8. Presence of the following chronic diseases:
    a. Cancer
    b. Hematological malignancy
    c. AIDS
    d. Diabetes mellitus
    e. COPD
    f. Asthma
    g. Liver cirrhosis
    h. Hypertension
    i. Coronary artery disease
    j. Congestive heart failure

   k. Peripheral vascular disease
   l. Dementia
   m. Prior stroke with sequelae
9. Survey of smoking history including:
   a. Ever smoker (> 100 cigarettes in lifetime)?
   b. If yes, current smoker?
      • Estimate of pack years (# packs per day) x (# years smoked)
   c. If former smoker, when did the subject quit smoking?
10. Pregnancy test (serum or urine) for women of childbearing potential

The following baseline clinical parameters will also be recorded. If more than one value is available for this 24-hour period, the appropriate values for the APACHE II calculator will be recorded. If no values are available from the 24 hours prior to study drug administration, then values must be measured prior to initiation of study drug. A full description of the below data collection is in **Section 8.1**.

Baseline Clinical Parameters

1. Vital Signs
2. CVP if available
3. Electrocardiogram (EKG)
4. Ventilator Parameters
5. Frontal Chest Radiograph (qualifying radiograph)
6. Lung Injury Score (LIS)
7. APACHE II Score
8. SOFA Score
9. Glasgow Coma Score (GCS)
10. Richmond Agitation Sedation Scale (RASS)
11. ABG (qualifying arterial blood gas)
12. SpO_2
13. $Scv0_2$ if available
14. Complete blood count (CBC)
15. Basic metabolic panel
16. PT/PTT/INR if available
17. Serum CK, AST, ALT, Albumin, Total Protein if available
18. Bilirubin
19. Lactate
20. Vasopressors and Inotropes Review
21. Fluid intake, fluid output (most recent 24 hour value) or mean hourly value for most recently available period
22. Renal replacement therapy status
23. Concomitant Medications Review
24. Discarded bronchoalveolar lavage (BAL) fluid and plasma if available

8.5 STUDY DRUG ADMINISTRATION DAYS 1 – 3

Study Day 1 will be within 24 hours of randomization. Prior to treatment on each study day, blood will be drawn for measurement of COHb level, complete blood count (CBC), lactate, blood gas analysis (either ABG or venous blood gas [VBG]), and additional laboratory values (as outlined in **Section 1.3**) if not ordered by the clinical team as part of routine ICU care. The subject's chart will be reviewed daily for

interval medical events. The subject's ventilator settings, vital signs, laboratory values, EKG, chest X-ray, and concomitant medications will be reviewed to determine if the subject remains eligible for treatment on each study day. Daily Hold Parameters are outlined in **Section 6.1.6.**

If the subject remains eligible for treatment, the study visit will require approximately 3 hours. Treatment will last 90 minutes with additional safety monitoring for an additional 90 minutes immediately following study drug completion. Blood will be drawn at serial time point during treatment and after treatment for measurement of COHb levels and blood gas analysis **(Table 5)**.

- Blood collection for COHb measurements pre-treatment and after 20 minutes for estimation of DLCO and determination of CFK-equation determined iCO dose to achieve a COHb level of 7% (target 6-8%) at 90 minutes (in subjects randomized to CO)
- Blood collection for COHb measurements at 40 minutes, 60 minutes, 75 minutes, 90 minutes, and 3 hours for safety monitoring
- Arterial or venous blood collection for blood gas analysis pre-treatment and at 90 minutes
- Blood collection for research labs (EDTA and PAXgene tubes) pre- and post-treatment (90 min, 3 hours)
- Urine collection for research labs pre- and post-treatment (90 min, 3 hours)

8.5.1 REFERENCE MEASUREMENTS (DAYS 1 – 3)

The following parameters will be measured and recorded daily on study Days 1 through 3 from 3:00-10:00 AM using the values closest to 8:00 am on the days specified in the **Schedule of Activities (Section 1.3)**. On days when the study drug is administered, the ventilator parameters will be recorded using the values closest to the study drug administration. Details regarding the below assessments are found in **Section 8.1.**

The following conditions will be ensured prior to reference measurements:

- No endobronchial suctioning for 10 minutes
- No invasive procedures or ventilator changes for 30 minutes
- All vascular pressures will be zero-referenced to the mid-axillary line with the patient supine.

Reference Measurements

1. Vital Signs
2. CVP if available
3. EKG
4. Ventilator Parameters Assessment (closest to the time of study drug administration)
5. Frontal Chest Radiograph when available
6. Lung Injury Score (LIS)
7. SOFA Score
8. GCS
9. RASS
10. $Scv0_2$ if available
11. CBC
12. Basic metabolic panel if available and requested by treating physician
13. PT/PTT/INR if available and requested by treating physician
14. Serum CK, AST, ALT, Albumin, Total Protein if available and requested by treating physician
15. Bilirubin
16. Lactate

17. Vasopressors and Inotropes Review
18. Fluid intake, fluid output (most recent 24 hour value) or mean hourly value for most recently available period
19. Renal replacement therapy status
20. Concomitant Medications Review
21. Adverse Event Monitoring
22. Blood sample collection for research labs
23. Urine sample collection for research labs
24. Microbiological results when available
25. Discarded BAL fluid and plasma if available

8.5.2 PRE-TREATMENT ASSESSMENTS (DAY 1 – 3)

The following assessments will be done on Days 1-3 prior to study drug administration. **No treatment will be started before these assessments are performed and the pre-treatment administration checklist is completed by the study investigator.**

- **Blood Gas Analysis (arterial or venous):** Arterial or venous blood will be drawn for blood gas analysis using the IL GEM Premier Co-oximeter or local laboratory at each center. The blood gas will be assessed daily prior to starting study drug administration. The study drug will not be started until this result is obtained and reviewed by the study physician investigator for a potential study hold. The following values will be recorded from the ABG or VBG results: pH, PCO_2, PO_2, and base excess or deficit.
- **Hemoglobin Level:** Hemoglobin will be measured and assessed daily prior to study drug administration. The hemoglobin level will be measured by the local laboratory at each center. The study drug will not be started until this result is obtained and reviewed by the study physician investigator for a potential study hold.
- **COHb Level:** The baseline COHb level will be drawn and assessed by an unblinded physician investigator, just prior (less than 15 minutes) to starting the study drug administration. COHb will be measured by study investigators using an IL GEM Premier Co-oximeter at the subject's bedside. These results are obtained within less than 5 minutes. The study drug will not be started until this result is obtained and reviewed for a potential study hold. All COHb level result printouts should be filed in the unblinded subject file.
- **Lactate Level:** Lactate level will be measured and assessed daily by the local laboratory at each center prior to study drug administration. The study drug will not be started until this result is obtained and reviewed by the study physician investigator for a potential study hold.
- **EKG:** A 12 lead EKG will be obtained and reviewed daily by the study staff physician prior to study drug administration to evaluate for cardiac exclusion criteria (acute MI, ACS, or unstable atrial or ventricular arrhythmia). The study drug will not be started until the EKG is obtained and reviewed by the study physician investigator for a potential study hold. The EKG will also be officially read locally at each site.
- **Calibration of IL GEM Premier Co-oximeter and COventDS:** The IL GEM Premier Co-oximeter and COventDS will be calibrated according to the Manual of Operations prior to study drug administration.
- **Calculate Deadspace (Vd/Vt):** Dead space fraction will be measured daily by the administering RT or physician prior to study drug administration using a NICO or NM3 monitor (Respironics) and the PCO_2 obtained from an arterial or venous blood gas.

- **Ambient Air CO Monitoring:** Ambient air CO levels will be measured before and throughout study drug treatment using a calibrated Dräger Pac 7000 CO detector. This will ensure that ambient levels are maintained within acceptable limits for occupational exposure.
- **Review of Reference Measurements:** The unblinded study investigator will review reference measurements outlined in **Section 8.5.1.**
- **Review of Adverse Events:** The blinded study coordinator will review events over the past 24 hours that may meet criteria for adverse events.
- **Review Daily Hold Parameters** (detailed information in **Section 6.1.6**) prior to starting Study Drug administration.

8.5.3 STUDY DRUG ADMINISTRATION AND MONITORING

The study drug will be administered for up to 90 minutes daily for up to 3 days following randomization or until discontinuation of mechanical ventilation, whichever occurs first according to **Section 6.1.9**. See **Sections 6.1.6, 6.1.7, and 6.1.8** for information about study drug hold, interruption, and permanent discontinuation criteria.

Study Drug Dosing:
Subjects randomized to CO will be administered iCO at 200 ppm for 20 min as a "test dose" on each treatment day to determine in real-time each subject's CFK equation-determined personalized dose. COHb levels will be measured before and after the 20 min test dose, and these two levels entered into the CFK equation calculator program to estimate DL_{CO} and compute the iCO dose to achieve a COHb level of 7% (target 6-8%) at 90 min. After the iCO dose is determined, subjects will be administered iCO at the CFK equation-determined personalized dose (200-500 ppm) but not to exceed 500 ppm or 90 min total (including test dose). Subjects randomized to placebo will be administered air.

Monitoring during Study Drug Administration:
Subjects will be monitored with continuous pulse oximetry, cardiac monitoring, and either intermittent (at least every 15 minutes) or continuous blood pressure monitoring (if an arterial line is in place) throughout the course of the 90-minute study drug treatment.

SpCO and SpO_2 will be monitored continuously using a Masimo Rad-7 non-invasive pulse oximeter, and recorded at the time points specified in **Table 5**. COHb levels will be measured using an IL GEM Premier Co-oximeter and recorded at the time points specified below. Arterial or venous blood will be drawn for blood gas analysis prior to treatment and at the completion of study drug administration (90 min or earlier if discontinued prior to 90 min). If study drug treatment is discontinued prior to 90 min, all of the scheduled 90-minute measurements will be made and recorded at the time of study drug discontinuation. Ambient CO levels will be monitored continuously using a Dräger Pac 7000 CO detector, and recorded at the time points specified below.

Table 5. CO Monitoring During Treatment

	ABG or VBG (pH, PCO_2, PO_2)	**FiO_2**	**COHb level** (IL GEM Premier co-oximeter)	**CO Dose Adjustment** (CFK calculator)	**SpCO** (Masimo Rad-7)	**SpO_2** (Masimo Rad-7)	**Ambient CO Level** (Dräger Pac CO detector)

Pre-treatment	X	X	X		X	X	X
20 min			X	X	X	X	X
40 min			X		X	X	X
60 min			X		X	X	X
75 min			X		X	X	X
90 min*	X	X	X		X	X	X
180 min			X		X	X	X

*90 min or upon completion of study product if discontinued sooner than 90 min.

8.6 POST-TREATMENT ASSESSMENTS

8.6.1 STUDY DAYS 4, 5, AND 7

If the subject remains hospitalized, the following assessments will be completed. See **Section 8.1** for descriptions of study assessments.

- Review interval medical events, ventilator settings, vital signs, and concomitant medications
- Review EKG on study day 7, and other days if available
- Review chest X-ray on study day 7, and other days when available
- Urine and blood collection for research labs on study day 5
- ABG or VBG when available
- SpO2
- Lung injury score (LIS)
- Dead space fraction (Vd/Vt) on day 7
- Obtain Glasgow Coma Score (GCS), SOFA score, and Richmond Agitation Sedation Scale (RASS)
- The following lab values will be recorded if available and requested by treating physician: CBC, basic metabolic panel, PT/PTT/INR, Serum CK, AST, ALT, Albumin, Total Protein, and Lactate
- Collection of blood for bilirubin if not drawn by the clinical team
- Vasopressors and inotropes review
- Fluid intake and output (most recent 24 hour value) or mean hourly value for most recently available period on days 4 and 5
- Renal Replacement Therapy Status
- Adverse event review
- Microbial results when available
- Discarded BAL and plasma samples when available

8.6.2 STUDY DAY 14

If the subject remains hospitalized, the following assessments will be completed. See **Section 8.1** for descriptions of study assessments.

- Ventilator Parameters Assessment
- SOFA Score
- GCS
- SpO2 when available
- Blood collection for total bilirubin if not drawn by the clinical team

- ABG or VBG when available
- CBC and basic metabolic panel when available
- Vasopressors and Inotropes Review
- Renal Replacement Therapy Status
- Microbiological results when available

8.6.3 STUDY DAY 28

If the subject remains hospitalized, the following assessments will be completed. See **Section 8.1** for descriptions of study assessments.

- Review interval medical events
- Obtain GCS and SOFA score at Day 28 or on discharge date
- Collection of blood for total bilirubin if not drawn by the clinical team
- CBC and basic metabolic panel if available
- Microbial results when available
- Vasopressors and Inotropes Review
- Renal Replacement Therapy Status
- Discharge, ventilator, and vasopressor status

If the subject has been discharged home or to a long-term acute care facility, the subject will be contacted by telephone to:

- Obtain vital status

8.6.4 STUDY DAY 60

If the subject remains hospitalized, the following assessments will be completed. See **Section 8.1** for descriptions of study assessments.

- Discharge, ventilator, and vasopressor status

If the subject has been discharged home or to a long-term acute care facility, the subject will be contacted by telephone to:

- Obtain vital status

8.6.5 MONTH 3 AND 6 ASSESSMENTS

Subjects will be contacted by phone at 3 and 6 months for the following assessments. See **Section 8.1** for descriptions of study assessments:

- Obtain vital status
- Neurocognitive testing- Telephone versions of the Montreal Cognitive Assessment (MoCA) and Hayling sentence completion test

8.7 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.7.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.7.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- results in death;
- is life threatening (places the subject at immediate risk of death from the event as it occurred);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above.

Organ failures related to ARDS or the patient's underlying condition that are systematically captured by the protocol should not be reported as adverse events ***unless they are considered to be study related*.** If the suspected adverse reaction is unexpected, it will be classified as a serious unexpected suspected adverse reaction (SUSAR).

8.7.3 DEFINITION OF ADMINISTRATION RELATED ADVERSE EVENTS

For this trial, a subset of adverse events will be considered to be "administration related adverse events". These "administration related adverse events" will by definition be considered suspected adverse reactions. These events are:

- Acute MI* within 48 hours of study drug administration
- Acute stroke** within 48 hours of study drug administration
- New onset atrial or ventricular arrhythmia requiring DC cardioversion within 48 hours of study drug administration
- Increased oxygenation requirements defined as: an increase in FiO_2 of ≥ 0.2 **AND** increase in PEEP ≥ 5 cm H_2O within 6 hours of study drug administration
- Increase in any protocol-specified measurement of COHb ≥ 10%
- Increase in lactate by ≥ 2 mmol/L within 6 hours of study drug administration

* Acute MI is defined below according to the Executive Group on behalf of the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC)/American Heart Association (AHA)/World Heart Federation (WHF) Task Force for the Universal Definition of Myocardial Infarction[144]. We will also rely upon subspecialty consultation to confirm the presence or absence of an acute MI.

Acute myocardial infarction is defined as acute myocardial injury with clinical evidence of acute myocardial ischemia and with detection of a rise and/or fall of cardiac troponin values with at least 1 value above the 99th percentile upper reference limit and at least 1 of the following:

- Symptoms of myocardial ischemia;
- New ischemic ECG changes;
- Development of pathological Q waves;

• Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischemic etiology;
• Identification of a coronary thrombus by angiography or autopsy (not for types 2 or 3 MIs).

** Acute stroke is defined as an acute neurological deficit attributed to an acute focal injury of the central nervous system by a vascular cause, including cerebral infarction, intracerebral hemorrhage, and subarachnoid hemorrhage based on neuropathological, neuroimaging, and/or other clinical evidence of permanent injury[145]. We will also rely upon subspecialty consultation to confirm the presence or absence of an acute stroke.

8.7.4 CLASSIFICATION OF AN ADVERSE EVENT

8.7.4.1 SEVERITY OF EVENT

For adverse events (AEs) not included in the protocol defined grading system, the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 guidelines will be used to describe severity.

- **Grade 1 Mild** – asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- **Grade 2 Moderate** – minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).
- **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** – urgent intervention indicated.
- **Grade 5 Death**

8.7.4.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) will have their relationship to study intervention assessed by a designated blinded study physician at each site. Additional AE adjudication will be provided by one of the Medical Monitors who will review all AEs in an unblinded fashion as is currently recommended by the FDA for SAEs (Guidance for Industry and Investigators Safety: Reporting Requirements for INDs and BA/BE Studies (Section VI.C); December 2012). The Medical Monitors at the CCC will work collaboratively with the reporting investigator to determine if a serious adverse event has a reasonable possibility of having been caused by the study drug or procedure.

The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events).
- **Probably not related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship

improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

Administration related adverse events, as outlined in **Section 8.7.3** of the protocol will always be considered suspected adverse reactions if they occur within protocol-defined timeframes **(Section 8.7.3)** of study drug exposure.

8.7.4.3 EXPECTEDNESS

The designated blinded study physician at each site and the Medical Monitor(s) will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study product as described in the Investigator's Brochure.

8.7.5 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

Blinded study coordinators at each site will collect adverse event information daily during the period from enrollment to study day 7. A blinded study physician who is designated at each site will review AE information collected by the blinded study coordinators and adjudicate any clinical adverse events that occur during the period from enrollment to study day 7. Adverse event collection begins after the patient or surrogate has signed informed consent and has received study drug or undergone study procedures. If a patient experiences an adverse event after consent, but prior to receiving study product, the event will NOT be collected unless the investigator feels the event may have been caused by a protocol procedure. The designated blinded study physician will evaluate any changes in laboratory values and physical signs and will determine if these changes are clinically important. All clinically important adverse events will be recorded in the case report form regardless of attribution to study product. After study day 7, adverse events are not required to be reported unless the investigator feels the events were related to either study product or a protocol procedure.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the pre-existing medical condition deteriorates at any time between the first study product administration and study day 7, it will be recorded as an AE. Organ failures related to ARDS or the patient's underlying condition that are systematically captured by the protocol should not be reported as adverse events ***unless they are considered to be study related*.**

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs will be followed for outcome information until resolution or stabilization or until 28 days (or hospital discharge whichever occurs first) after enrollment.

For this trial, a subset of adverse events will be considered to be "administration related adverse events". These "administration related adverse events" will by definition be considered suspected adverse reactions. These events are:

- Acute MI within 48 hours of study drug administration
- Acute stroke within 48 hours of study drug administration
- New onset atrial or ventricular arrhythmia requiring DC cardioversion within 48 hours of study drug administration
- Increased oxygenation requirements defined as: an increase in FiO_2 of ≥ 0.2 **AND** increase in PEEP ≥ 5 cm H_2O within 6 hours of study drug administration
- Increase in any protocol-specified measurement of COHb ≥ 10%
- Increase in lactate by ≥ 2 mmol/L within 6 hours of study drug administration

8.7.6 ADVERSE EVENT REPORTING

All clinically important AEs as described in **Section 8.7.5**, will be recorded in the StudyTRAX database. For each AE, an email will be sent to the CCC, site PI, and blinded physician adjudicating AEs at each site. AEs will be summarized using System Organ Class and preferred terms. Tables will show by treatment group the number and percentage of participants experiencing an AE within a System Organ Class or within preferred term category by severity or by relationship to study product. For the calculations in these tables, a participant with multiple AEs within a category will be counted once under the maximum severity or the strongest recorded causal relationship to study product. Formal statistical testing comparing groups is not planned since interpretation of differences must rely heavily upon clinical judgment.

8.7.7 SERIOUS ADVERSE EVENT REPORTING

Site investigators will report all **serious** AND **unexpected adverse events or reactions**, regardless of relationship to the study intervention, to the CCC by entering the event information into StudyTrax within 24 hours of becoming aware of the event. StudyTrax will automatically send an email alert to the CCC and DCC study leadership. The site investigator should include an assessment of whether there is a reasonable possibility that the study intervention caused the event.

Investigators will also report all **serious** AND **administration related adverse events** (as described in **Section 8.7.3**) to the CCC by email within 24 hours of becoming aware of the event. An email will also be sent from the StudyTRAX database alerting the CCC to the event. The CCC will review the event and may inform the site to permanently discontinue study drug administration to the subject **(Section 6.1.8)**, and may hold enrollment pending Executive Committee (EC) and DSMB review **(Section 10.1.6)**. The CCC may also request additional information from the site investigator.

If the event is characterized as ***unexpected* and related or *possibly related to the research*** and indicates that there are new or increased risks to subjects, the CCC will notify the MGB IRB within 5 working days/7 calendar days of the date the investigator first becomes aware of the problem according to the MGB IRB requirements.

The CCC will report all **unexpected and study-related deaths** or **life-threatening suspected serious adverse events (SUSAR)** to the FDA within 7 calendar days of receipt. The CCC will report **all deaths occurring during the study hospitalization** and **all serious, unexpected, and study-related adverse events** and **all administration related adverse events** to the DSMB, by email, or telephone, within 7 calendar days of the CCC being notified of the event. A written report will be sent to the DSMB and the FDA within 15 calendar days. Site investigators will also be sent these reports and will follow their local IRB's policy for reporting.

8.8 UNANTICIPATED PROBLEMS

8.8.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

An unanticipated problem involving risks to participants or others is defined as any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.8.2 UNANTICIPATED PROBLEM REPORTING

Site investigators will report unanticipated problems (UPs) to the PI and CCC within 24 hours. Unanticipated problems will be reported to the sIRB within 5 working days/7 calendar days of the date the investigator first becomes aware of the problem. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Hypothesis: We hypothesize that low dose iCO will be safe and well-tolerated and that the CFK equation-based iCO personalized dosing algorithm will be accurate in achieving a target COHb level of 6-8% in patients pneumonia (without ARDS and sepsis or with sepsis without ARDS criteria) and with sepsis-induced ARDS at study entry.

We further hypothesize that precision-based iCO therapy will reduce the severity of pneumonia and ARDS and organ failure by suppressing mitochondrial dysfunction, inhibiting inflammasome activation and necroptosis, and accelerating resolution of inflammation.

Primary Endpoints:

1. The primary endpoint is **safety of iCO**, defined by the incidence of pre-specified administration-related AEs and SAEs.

   Administration-related SAEs are: 1) myocardial infarction; 2) stroke; 3) new onset arrhythmia requiring DC cardioversion; 4) worsening hypoxemia[1,2]; 5) COHb ≥ 10%; and 6) increase in lactate by ≥ 2 mmol/L.

2. **Accuracy** of the CFK equation-based personalized iCO dosing algorithm to achieve a COHb level of 6-8%.

Secondary Endpoints:

1. Lung Injury Score (LIS) on days 1-5 and day 7
2. PaO2/FiO2 ratio on days 1-5 and day 7
3. Oxygenation index (OI) on days 1-5, and 7
4. Dead space fraction (Vd/Vt) on days 1-3 and day 7
5. Sequential Organ Failure Assessment (SOFA) scores on days 1-5, 7, 14, and 28
6. Ventilator-free days at day 28
7. ICU-free days at day 28
8. Hospital-free days to day 60
9. Hospital mortality to days 28 and 60
10. Neurocognitive function at 3 and 6 months

Exploratory Endpoints:

1. Plasma and urine biomarkers of mitochondrial dysfunction (mtDNA)
2. Plasma and urine biomarkers of inflammasome activation (IL-18)
3. Plasma and urine biomarkers of necroptosis (RIPK3)
4. Plasma Lipid mediators (LM) and specialized pro-resolving mediators (SPMs)

9.2 SAMPLE SIZE DETERMINATION

As the primary endpoint of this Phase Ib trial is safety, this sample size of 36 (2:1 CO:placebo) was chosen based on a probability to estimate the mean difference between measured and predicted COHb levels in the CO group within 0.5% with 95% confidence to support the accuracy of the CFK equation.

Our preliminary data from our Phase Ia trial demonstrate that the CFK equation is highly accurate at predicting COHb levels following a 20 minute exposure to 200 ppm iCO ($r=0.916$, $R^2=0.9204$; $p<0.0001$). The mean difference between measured and predicted COHb levels was 0.1467 ± 0.2738. Using a more conservative estimate of standard deviation (SD)=0.6, we will have >99% probability that 95% confidence intervals around the mean difference between measured and predicted COHb levels will be -0.353% to 0.647%. Our calculation includes an 8% dropout rate.

The goal of the safety evaluation for this study is to identify safety concerns associated with CO administration. The ability of the study to detect administration-related AEs or SAEs can be expressed by the true event rate above which at least one SAE would likely be observed and the true event rate below which no events would likely be observed. Specifically, for the CO arm of the study (n=24), there is a 90% probability of observing at least one AE if the true background rate of that AE is 10% and a 97% probability of observing at least one AE if the true background rate of that AE is 15%. For the placebo arm of the study (n=12), there is an 83% probability of observing at least one AE if the true background rate of that AE is 15% and a 91% probability of observing at least one AE if the true background rate of that AE is 20%. In our Phase Ia trial, there were no administration-related AEs and no SAEs related to study product or procedures.

Probabilities of observing one or more events between the groups are presented in **Table 6** below for a range of possible true AE rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with CO.

Table 6. Probability of observing 1 or more events between the groups for different true AE rates

CO group

n	True AE rate	Power to detect ≥1 AE
24	0.010	0.20
24	0.020	0.36
24	0.035	0.54
24	0.050	0.68
24	0.100	0.90
24	0.150	0.97
24	0.200	0.99

Placebo Group

N	True AE rate	Power to detect ≥1 AE
12	0.010	0.10
12	0.020	0.20
12	0.035	0.32
12	0.050	0.43
12	0.100	0.69
12	0.150	0.83
12	0.200	0.91

9.3 POPULATIONS FOR ANALYSES

All randomized participants will be included in the intent-to-treat (ITT) population. All randomized subjects who receive at least one dose of study product (CO vs. placebo) will be included in the safety analyses (*i.e.*. modified ITT). For secondary and exploratory endpoints, subjects may also be analyzed according to the number of study drug doses completed.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

We will use the Mann-Whitney U or t-test, chi-square (χ^2) or Fisher's exact tests to analyze baseline demographics and clinical characteristics between the treatment groups. Distribution of numerical outcomes will be evaluated prior to any analysis. Numerical outcomes will be compared between treatment arms using the Mann-Whitney U or t-test and categorical outcomes compared using either χ^2 or Fisher's exact test.

9.4.2 ANALYSIS OF THE PRIMARY SAFETY ENDPOINT(S)

We hypothesize that iCO will be safe and well-tolerated and that our iCO dosing algorithm will be accurate

in achieving target COHb levels.

We will examine all AEs including pre-specified administration-related AEs by the type of event, as well as by body system class, and will report proportions and 95% CI. We will compare the occurrence of specific AEs in each arm by the χ^2 test, and the number of AEs per patient in each arm by the Wilcoxon-Mann-Whitney test.

COHb levels will be summarized descriptively reporting mean, standard deviation, median, minimum, and maximum at each time point, each day, by treatment arm. The mean difference between predicted and measured COHb levels with 95% confidence intervals will be presented for the iCO group. Repeated measures analysis of variance (ANOVA) will be used to assess the effects of treatment, time (over 3 days), and the interaction between time and treatment for 90-minute COHb levels. ANOVA will be repeated to assess the effects of treatment, time (over 3 days), entry criteria (ARDS/Sepsis vs. Non-ARDS/Sepsis Pneumonia vs. Non-ARDS/non-Sepsis Pneumonia), and two-way/three-way interactions of time, treatment and entry criteria. We will also compare the occurrence of COHb ≥ 10% in each arm by the χ^2 test.

Accuracy of the CFK equation-based personalized iCO dosing algorithm to achieve target COHb levels will also be analyzed by Spearman correlation and Bland-Altman plots using measured vs. predicted COHb levels and modeled using linear or non-linear regression.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

We also hypothesize that iCO will reduce the severity of ARDS as measured by lung injury score (LIS), PaO_2/FiO_2, oxygenation index (OI), and dead space fraction (Vd/Vt). These endpoints will be analyzed using repeated measures analysis of covariance (ANCOVA) to assess the effects of treatment, time, and the interaction between time and treatment, while incorporating between-patient variation in baseline measurements and important covariates including smoking, age, sex, and severity of ARDS. Age and sex will be considered as important biological variables in all analyses.

Interaction contrasts will include polynomial trends across time, focusing on a linear trend suggesting improvement and a quadratic trend suggesting that improvement is leveling off. As part of our analysis, we will also include the interaction between treatment and entry criteria (ARDS/Sepsis vs. Non-ARDS/Sepsis Pneumonia vs. Non-ARDS/non-Sepsis Pneumonia)in the model. Because the study is not powered for the interaction between treatment and entry criteria, that test will be exploratory. We will also perform post-hoc analyses within each criteria group to generate hypotheses for future study.

With 24 subjects in the CO group and 12 subjects in the placebo arm, we will have 80% power (α=0.05) to detect a 0.95 difference in LIS between treatment arms, assuming a conservative SD of 0.8 **(Table 7)**. We will also have 80% power to detect a 78.6 mm Hg difference in PaO_2/FiO_2, a 12.0 difference in OI, and a 0.14 difference in Vd/Vt between treatment arms, assuming SDs of 66, 10.1, and 0.12 respectively.

Table 7. Minimal detectable difference at 80% power.

Endpoint	SD	Detectable Difference (α= 0.05)
LIS	0.8	0.95
PaO_2/FiO_2	66	78.6

Endpoint	SD	Detectable Difference (α= 0.05)
OI	10.1	12.0
Vd/Vt	0.12	0.14

Ventilator-free days (VFDs), hospital- and ICU-free days, and Sequential Organ Failure Assessment (SOFA) scores will each be analyzed separately using ANCOVA with treatment arm as the main effect. Given our modest sample size, we will only be powered to see large changes.

Results from the Hayling Sentence Completion Test and Montreal Cognitive Assessment (MoCA) testing will be compared between CO and placebo groups using the Mann-Whitney U test or t-test (if normally distributed). If significantly different between the groups, scores from the Hayling Sentence Completion Test and MoCA testing may be incorporated into exploratory multiple regression analyses.

Mortality will be analyzed using Cox regression models for time-to-death with treatment arm as the main predictor, and with important covariates including smoking, age, and sex in the model. Hazard ratio (HR) and 95% CI for CO relative to placebo will be reported, along with Kaplan-Meier plots and estimated proportions surviving at 28, 60, and 180 days.

To address potential bias from unobserved study outcomes due to missed visits, premature withdrawal, or death and to ensure robust data analyses, we will work closely with Dr. Dale Needham at Johns Hopkins University, who has extensive expertise with long-term outcomes assessment after acute respiratory failure and critical illness. Dr. Needham will provide his expertise and assist with statistical methods developed by his Outcomes After Critical Illness and Surgery (OACIS) Group to address missing data and assess long-term outcomes that may be “truncated due to death”. We will use the statistical software application developed by his group using R and Shiny to impute missing data among survivors given the high mortality in ARDS. We will also employ other statistical approaches used by his OACIS Group to assess long-term outcomes in critically ill patients. These include a composite endpoint approach using both functional outcome and survival, as well as other statistical methods (*e*.g. survivors only analyses and survivor average causal effect [SACE]).

9.4.4 ANALYSIS OF EXPLORATORY ENDPOINTS (BIOMARKERS)

We will compare biological outcomes between treatment arms using the Mann-Whitney U or t-test for numerical endpoints and either χ^2 or Fisher’s exact test for categorical outcomes. Lipid mediators (LM) and specialized pro-resolving mediators (SPM) levels will be analyzed using principal component analysis (PCA).

We hypothesize that treatment with iCO will reduce plasma levels of mitochondrial DNA (mtDNA), IL-18, and receptor-interacting serine/threonine-protein kinase 3 (RIPK3). Circulating IL-18, mtDNA, and RIPK3 levels will be log transformed before analysis. Based on our prior work, the SD of the log-IL-18 is approximately 0.7, approximately 1.0 for mtDNA, and approximately 2.0 for RIPK3. With 12 subjects in the placebo arm and 24 in the iCO arm, we will have 80% power to detect a difference in IL-18 levels of 0.834 log, which corresponds to an approximate 2-fold difference in plasma IL-18. We will have 80% power to detect a difference in mtDNA of 1.190 log, which corresponds to an approximate 3-fold difference in the circulating mtDNA copy number between the iCO and placebo groups.

We will also have 80% power to detect a difference in RIPK3 of 2.38 log, which corresponds to an approximate ~~8~~ 10-fold difference in the circulating RIPK3 levels between the iCO and placebo groups. While the magnitude of the putative CO effect on these 3 biomarkers is unknown, we found a 3-fold difference in IL-18 between sepsis patients with and without ARDS and a 4-fold difference in mtDNA between sepsis/ARDS patients and controls. Similarly, we found a 7-fold difference in median RIP3K levels between patients with organ failure and shock compared with ICU patients without organ failure or shock. Therefore detecting differences of these magnitudes is feasible in this patient population. Based on our preliminary data, we will also have 80% power (α=0.05) to detect a difference in a number of LM and SPM including PGD_2, PGE_2, $PGF_{2\alpha}$, TXB_2, RvD2, and Mar1 **(Table 8)**.

Table 8. Minimal detectable difference at 80% power.

	Mediator	Standard Deviation	Detectable Difference (pg/mL)
Pro-inflammatory LM	LTB_4	17.95	21.4
	PGD_2	0.68	0.81
	PGE_2	1.68	2.00
	$PGF_{2\alpha}$	1.15	1.37
	TXB_2	12.38	14.75
Pro-resolving SPM	RVD_2	12.18	14.5
	Mar1	15.29	18.2
	RvE1	10.54	12.55

We will assess correlations between biomarkers and clinical and lung physiology outcomes including LIS, SOFA, VFDs, as well as 28 and 60 day hospital mortality. We will use multivariable linear regression as the primary model with LIS as the primary outcome variable. Biomarker levels tend to exhibit right skewedness and large outliers, so data will likely require log transformation. Additional predictor variables (such as demographic variables and comorbidities) will be specified *a priori* and interactions between those variables and biomarkers will be included in the model with adjustment for age and sex as important biological variables. To evaluate the predictive ability of biomarkers for binary outcomes, we will use two methods. We will use c-statistics obtained from a logistic regression model to evaluate the effect of biomarker measurements on increasing the area under the receiver operating characteristic (ROC) curves. In addition, we will test if including measured biomarkers to models including important covariates results in an improvement in the net reclassification index as we have done previously for mtDNA.

9.4.5 SAFETY ANALYSES

The primary endpoint is safety of iCO, defined by the incidence of pre-specified administration-related AEs and SAEs. Administration-related SAEs are: 1) myocardial infarction; 2) stroke; 3) new onset arrhythmia requiring DC cardioversion; 4) worsening hypoxemia[1,2]; 5) COHb ≥ 10%; and 6) increase in lactate by ≥ 2 mmol/L. The primary endpoint is also accuracy of the CFK equation-based personalized iCO dosing algorithm to achieve a COHb level of 6-8%.

We will examine all AEs including pre-specified administration-related AEs by the type of event, as well as by body system class, and will report proportions and 95% CI. We will compare the occurrence of specific AEs in each arm by the χ^2 test, and the number of AEs per patient in each arm by the Wilcoxon-Mann-Whitney test.

For AEs not included in the protocol defined grading system, the CTCAE Version 5.0 guidelines will be used to describe severity. The CTCAE term, start and stop date, severity, relationship, expectedness, and outcome will be reported for all AEs. A separate table will report the incidence of pre-specified administration-related AEs per arm.

COHb levels will be summarized descriptively reporting mean, standard deviation, median, minimum, and maximum at each time point, each day, by treatment arm. The mean difference between predicted and measured COHb levels with 95% confidence intervals will be presented for the iCO group. Repeated measures analysis of variance (ANOVA) will be used to assess the effects of treatment, time (over 3 days), and the interaction between time and treatment for 90-minute COHb levels. ANOVA will be repeated to assess the effects of treatment, time (over 3 days), entry criteria (ARDS/Sepsis vs. Non-ARDS/Sepsis Pneumonia vs. Non-ARDS/non-Sepsis Pneumonia), and two-way/three-way interactions of time, treatment and entry criteria. We will also compare the occurrence of COHb ≥ 10% in each arm by the χ^2 test.

Accuracy of the CFK equation-based personalized iCO dosing algorithm to achieve target COHb levels will also be analyzed by Spearman correlation and Bland-Altman plots using measured vs. predicted COHb levels and modeled using linear or non-linear regression.

9.4.6 PLANNED INTERIM ANALYSES

Safety monitoring will be conducted on a regular basis by the EC and DSMB. The EC will independently review the safety data in a blinded fashion at regular intervals and make recommendations to the DSMB and NHLBI. The DSMB will meet at least semiannually or after enrollment of 12 subjects, whichever is sooner, to assess safety data on each arm of the study in an unblinded fashion. In addition, all related SAEs will be reported to the DSMB in an expedited fashion and the DSMB would have the option after every SAE report to request an *ad hoc* meeting if safety concerns arise. At this *ad hoc* meeting, the CCC would update the tabulations of all AEs and administration related adverse reactions for Board review. Recommendations by the EC and/or DSMB to amend the protocol or add additional subjects will be reviewed by the sIRB prior to implementation. The EC and/or DSMB may halt enrollment in the study at any time during the trial.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Federal regulations 45 CFR 46.111(a)(5) require that informed consent will be sought from each prospective subject or the subject's legally authorized representative (LAR). Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant/LAR and written documentation of informed consent is required prior to starting intervention/administering study intervention.

The investigator is responsible for ensuring that the patient/LAR understands the risks and benefits of participating in the study, and answering any questions the patient/LAR may have throughout the study and sharing any new information in a timely manner that may be relevant to the patient's willingness to continue his or her participation in the trial. All study participants or their surrogates will be informed of the objectives of the study and the potential risks. The informed consent document will be used to explain the risks and benefits of study participation to the patient in simple terms before the patient is entered into the study, and to document that the patient is satisfied with his or her understanding of the risks and benefits of participating in the study and desires to participate in the study. The investigator is responsible for ensuring that informed consent is given by each patient or legal representative. This includes obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures and prior to the administration of study agent.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

A licensed physician investigator will obtain written informed consent after a detailed review of the consent form with the patient, or more likely, the LAR of the potential study participant. The majority of the patients approached for participation in this study will have limitations of decision-making abilities due to their critical illness or need for sedating medications. Hence, most patients will not be able to provide informed consent. Accordingly, informed consent will be sought from the potential subject's LAR.

The physician investigator will be responsible for ensuring that the subject or LAR understands the risks and benefits of participating in the study, and answering any questions the subject or LAR may have throughout the study and sharing in a timely manner any new information that may be relevant to the subject's willingness to continue his or her participation in the trial. All study participants or their surrogates will be informed of the objectives of the study and the potential risks.

The informed consent document will be used to explain the risks and benefits of study participation to the patient or LAR in simple terms before the patient is entered into the study, and to document that the patient or LAR is satisfied with his or her understanding of the risks and benefits of participating in the study and desires to participate in the study. The investigator is responsible for ensuring that informed consent is given by each patient or legal representative. This includes obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures and prior to the administration of the study agent.

The subject or LAR will be given ample opportunity to read the consent form and ask questions during the initial consent visit and afterwards prior to the signing of the consent form. The subject or LAR will be allowed to discuss the study with anyone they choose before making a decision. The physician investigator obtaining informed consent will explain to the subject or LAR that questions can be addressed at any point throughout the trial and the PI's contact information will be made available during the consent visit for any questions that arise prior to or throughout the study. The subject or LAR will be given a signed copy of the informed consent form, which will include the site PI contact information and the subject or LAR will be encouraged to call with any questions.

For subjects for whom consent was initially obtained from a surrogate, but who subsequently regain decision-making capacity while in hospital, we will obtain formal consent for continuing participation, inclusive of continuance of data acquisition. The initial consent form signed by the surrogate will reflect that such continuing consent will be obtained when possible.

The following categories of surrogates (listed in general order of preference) may provide written informed consent on behalf of potential subjects incapable of providing informed consent themselves, however the exact order will be dependent on the sIRB and local IRB requirements at each site when relying on an sIRB.

- court appointed guardian with specific authority to consent to participation in research or authority to make health care decisions for a class of diagnostic and therapeutic decisions inclusive of the proposed research;
- health care proxy/person with durable power of attorney with specific authority for making health care decisions inclusive of the proposed research; or
- spouse, adult child, or other close family member who knows the subject well and has been involved in their care.

Subjects and/or surrogates will be given a written copy of the consent form, as well as ample time to read and understand the written material. The surrogate will be asked to consider the potential subject's own views prior to providing surrogate consent. An investigator will be available to answer any additional questions. Subjects and/or their surrogates can withdraw consent at any time.

If consent from a LAR or surrogate cannot be obtained in person on behalf of a subject with impaired decision-making capacity, a licensed physician investigator may call the subject's LAR to perform consent by phone according in accordance with the sIRB and each site's IRB policy. Consent obtained by telephone must comply with all regulatory requirements about the process, the consent elements, and documentation of consent.

For subjects for whom consent was initially obtained from a surrogate, but who subsequently regain decision-making capacity while in the hospital, we will obtain formal consent for continuing participation, inclusive of continuance of data acquisition. The initial consent form signed by the surrogate will reflect that such continuing consent will be obtained when possible.

After the patient or his/her legal representative has consented to the study, a copy of the signed informed consent form will also be included in his or her medical record. A note will be included in the medical chart to make other staff aware that the patient was enrolled in the study. The pager number of the PI or Co-investigator will be included in the note to allow the clinical staff to contact the research team at any time.

Remote Consent During COVID-19 Pandemic

Due to infection control measures and hospital visitor policy restrictions during the COVID-19 pandemic, it may not be possible for physician investigators to obtain wet consent from the subject/LAR in person. If unable to obtain wet consent from the subject's LAR in person, the physician investigator will obtain consent remotely via telephone call in accordance with the sIRB and each site's IRB policy and FDA guidance on the conduct of clinical trials during the COVID-19 pandemic. The consent form will be provided to the LAR via secure email or other electronic means including REDCap, Adobe Sign, DocuSign and asked to electronically sign the document, scan or take a picture of the consent signature page, and then return electronically to the research team, in accordance with the sIRB and each relying site's institutional policies.

The physician investigator will electronically sign or print and sign their own copy of the paper consent form and document the consent process in a note to file or informed consent process checklist. Documentation will include that the consent form was provided to the LAR, consent was obtained, the

method used to obtain consent, that the LAR signed a separate copy of the consent form, and the date/time of consent. A compiled copy of the consent including the document with the physician investigator's signature and the LAR's signature consent document will be retained in the study record and a copy of the compiled consent form will be uploaded to subject's medical record. Consent obtained electronically must comply with all regulatory requirements about the process, the consent elements, and documentation of consent.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping

Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigators, NIH, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the sIRB and site IRBs as required, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

If the trial is suspended, all enrollments and study product administration will cease, but other study procedures will continue. The EC and DSMB will convene for a conference call within seven days of the suspension to determine whether the suspension can be lifted or permanent discontinuation of study product administration is appropriate. Lifting the suspension requires the unanimous approval of all members of the EC and DSMB.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor. All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

All subjects or surrogates must provide written informed consent and signed HIPAA authorization prior to the performance of any screening or main study procedures.

Subject confidentiality will be protected throughout the study and no subject-identifying information will be released to anyone outside the IRB-approved study. Confidentiality will be secured through several mechanisms. To maintain confidentiality, all research specimens, data collection forms, and study reports will be identified only by a coded number. The coded subject identification number will be automatically generated as a consecutive number by the StudyTRAX database and only IRB-approved study staff will have access to the codes.

Investigators and study staff at each site will retain a Subject Code Key linking the subject ID number to the subject's identifying information. The Subject Code Key will always be kept secure and stored electronically on each site's approved storage approved for Confidential Data. All paper records linking the subject to their study ID code will be kept in a locked cabinet within a locked office. Any study forms and paper records containing personal identifier information (e.g., address, phone number) will be stored securely in a locked cabinet within a locked office at each study site.

Clinical data abstracted from the medical record will be entered into a study-specific database in StudyTRAX, a secure, web-based, data management tool which is US FDA Title 21 CFR Part 11 compliant. StudyTRAX hosted projects are fully HIPAA compliant and ensure access control, audit control, data integrity, user authentication, and transmission security. The StudyTRAX database will only be accessible to the IRB-approved study staff. Study participants will be identified only by unique study identification numbers. Other than dates related to an individual (e.g., Date of Birth, Date of Informed Consent, admission date, test date, discharge date, death date, etc.) and ages over 89 (as applicable), no patient HIPAA identifiers will be entered or inputted into the StudyTRAX EDC system database. Each participating site will have access to only its own site's data, with the exception of the study monitors, CCC, and DCC who will have access to data entered from all sites for preparation of study-related reports (DSMB, adverse event reporting), as well as for analysis of the study data/endpoints.

Research data will be maintained by and accessible only to IRB-approved study staff and investigators. Any paper records including informed consent forms, contact information etc., and/or physical data will be stored securely in a locked file cabinet in a locked office in a secure area at each site. Any electronic data stored by each site (e.g., screening log, patient name, MRN, contact information) will utilize only site-approved methods for Confidential Data. Access to data will be role based and minimum necessary access will be provisioned. Only de-identified data will be used in any data analysis.

Access to subject data and information at the study sites, including biological samples, will be restricted to IRB-approved authorized personnel. All study data analyses will be done with coded numbers only. Subjects will not be identified by name in any reports or publications, nor will the data be presented in such a way that the identity of individual subjects can be inferred. Clinical information will not be released without the written permission of the subject, except as necessary for monitoring by the CCC, DCC, sIRB, NHLBI, FDA or other authorized federal agencies. All data released by the DCC for studies including genetic studies will be de-identified. Should subjects or surrogates revoke their consent for genetic testing, the clinical sites will notify the DCC and all samples collected for genetic analysis for that subject will be destroyed. Confirmation of destruction of samples will be forwarded from the DCC to the clinical site.

Investigators will obtain consent to use social security numbers (SSN) to determine vital status of participants lost to follow-up. Contact information and the subject's SSN will be collected on paper at the time of consent. Contact information and SSN will be maintained on paper locally and will not appear in the study database.

Certificate of Confidentiality

To further protect the privacy of study participants, this research is protected by a Certificate of Confidentiality from the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

With the participant's approval and as approved by local IRBs, coded biological samples will be stored at a central repository located at BWH, along with coded study data, for use by other researchers including those outside of the study. These samples could be used for research related to carbon monoxide, ARDS, critical illness or other lung diseases. The central repository at BWH will also be provided with a code that will allow linking the biological specimens with the data from each participant, maintaining the blinding of the identity of the participant. Only the originating clinical trial site will be able to link coded samples or data to specific individuals.

Samples may also be stored in the central repository at BWH for future genetic research related to carbon monoxide, ARDS, critical illness or other lung diseases. Specific consent for future genetic research will be required.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regards to biosample storage may not be possible after the study is completed.

Datasets and associated documentation from the clinical trial will be submitted to the NHLBI data repository Biological Specimen and Data Repository Information Coordinating Center (BioLINCC). The datasets will be prepared in accordance with the NHLBI Policy for Data Sharing from Clinical Trials and Epidemiological Studies, and in accordance with the Guidelines for NHLBI Data Set Preparation. In addition to the clinical data from the trial, biomarker results will also be deposited in BioLINCC. The DCC will prepare the datasets in a manner that protects the privacy of study participants and will document the methods used to prepare the datasets to protect participant privacy before submission to the NHLBI data repository. To maintain participant confidentiality, the DCC will remove obvious identifiers and de-identify protected information by re-coding data in order to mask subject IDs and center identifiers.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator
Rebecca Baron, MD
Brigham and Women's Hospital
75 Francis Street, Boston, MA
Tel: 617-525-6642
Email: rbaron@bwh.harvard.edu

Medical Monitor	**Medical Monitor**
B. Taylor Thompson, MD	Crystal North, MD
Massachusetts General Hospital	Massachusetts General Hospital
55 Fruit Street, Boston, MA	55 Fruit Street, Boston, MA
Tel: 617-643-2411	Tel: 617-726-1721
E-mail: tthompson1@mgh.harvard.edu	Email: *cnorth@mgh.harvard.edu*

Medical Monitors

The unblinded Medical Monitors will oversee regulatory reporting for the trial. They will review all potential study participants with site investigators and will be available for site questions that arise regarding safety and eligibility throughout the study. The Medical Monitors will review all AEs and prepare reports for all pre-specified administration-related AEs and serious treatment-related AEs for expedited DSMB review. They will also prepare IND safety reports for all serious, unexpected, suspected adverse reactions (SUSARs) for expedited DSMB and FDA review.

Clinical Coordinating Center

The CCC will serve as the overall coordinating center for all study sites and will be directed by the Medical Monitors. The CCC will be responsible for the oversight of the conduct of the Phase Ib safety trial including design and implementation of the protocol, comprehensive training of site personnel, communication and coordination among all sites, AE reporting, and report generation for the DSMB, FDA, sIRB, and NHLBI in conjunction with the DCC. The CCC will train clinical trial personnel and serve as the central site to maintain the necessary materials to conduct the trial including standard operating procedures (SOPs), electronic Case Report Forms (CRFs), training documents, Site Performance Plans, Recruitment and Retention Plans, the Investigator's Brochure (IB), AE Reporting Guidelines, the DSMB Charter, the Manual of Operations, CO administration procedures, and long term neurocognitive outcomes assessment.

Data Coordinating Center

The DCC will oversee the overall data management and biostatistical support for the study, and will be directed by Dr. Karla Ballman at WCM. In conjunction with the CCC, the DCC will be responsible for the statistical design of the trial, randomization, data collection and monitoring, quality control and completeness of study data, interim monitoring of the data, biostatistical support and data analysis, and report generation for the DSMB, FDA, sIRB, and NHLBI. The DCC will oversee the Data Completeness and Quality Monitoring Reporting Plan, and will ensure completeness of subject assessment and data collection, complete data cleaning, and perform primary and secondary data analyses to ensure timely publication and dissemination of the study results. In conjunction with the CCC, the DCC will ensure that the study results are reported to ClinicalTrials.gov within 12 months of the primary completion date and will implement the data sharing plan, including submission of study data to BioLINCC. Together, the DCC and CCC will work closely together to ensure the successful completion of the Phase Ib clinical trial and timely dissemination of its results.

Executive Committee

The Executive Committee (EC) will be comprised of Dr. Choi, Dr. Baron, CCC Directors, IND Sponsor, and the DCC study statistician. Dr. Choi and Dr. Baron will serve as heads of the overall EC. They will lead videoconferences to provide the structure to facilitate rapid decision-making and contingency planning for study implementation and execution. The EC will also ensure rapid communication among the leadership group, with administrative support from the CCC Project Managers for study-related clinical

and scientific issues, as well as operational issues that may arise. A major role of the EC will be to independently review the safety data in a blinded fashion at regular intervals and make recommendations to the DSMB and NHLBI.

Steering Committee

The PIs Dr. Choi and Dr. Baron will assume primary responsibility for the overall administration of the project, providing scientific, administrative, and fiscal oversight. The Steering Committee will include the site PIs from each enrolling center. They will work closely with the EC to provide formal guidance to the project, with interactive input from the Steering Committee. The Steering Committee (SC) will provide support, advice, and guidance for the study.

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertise. Per NHLBI Policy for Data and Safety Monitoring of Extramural Clinical Studies, NHLBI may suggest DSMB members for this trial. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet at least semiannually or after enrollment of 12 subjects, whichever is sooner, and on as needed basis to assess safety data on each arm of the study. In addition, all related SAEs will be reported to the DSMB in an expedited fashion and the DSMB would have the option after every SAE report to request an *ad hoc* meeting if safety concerns arise. At this *ad hoc* meeting, the CCC would update the tabulations of all AEs and administration related adverse reactions for Board review. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. DSMB meetings may be held by conference call or face-to-face meeting.

The DSMB serves in an advisory capacity to the IND Sponsor. They will review the study protocol, monitor all aspects of the study (e.g., recruitment, adverse events, protocol adherence, data quality, attrition, demographic and baseline characteristics) and recommend protocol modifications, including early study termination. All proposed changes to the study protocol will be reviewed by the DSMB and the single IRB.

Data and Safety Monitoring Board (DSMB)

The principal role of the DSMB is to regularly monitor data from this trial, review and assess the performance of its operations, and make recommendations, as appropriate, to the Study PI, IND Sponsor, and Project PI with respect to:

- Benefit/risk ratio of procedures and participant burden
- Selection, recruitment, and retention of participants
- Adherence to protocol requirements
- Completeness, quality, and analysis of measurements
- Amendments to the study protocol and consent forms
- Performance of individual centers and core labs
- Participant safety
- Notification of and referral for adverse events

The Study PI and the CCC physician members will be responsible for the preparation of DSMB reports. Recommendations to end, modify, or continue the trial will be prepared by the DSMB executive secretary

for review by the Study PI and IND Sponsor. The Study PI and IND Sponsor will act on recommendations expeditiously in consultations with the Executive Committee. The executive secretary of the DSMB will be responsible for preparing the minutes for each meeting or conference call. DSMB recommendations will be distributed to all study investigators.

Single IRB

This study will use a single a sIRB to conduct the ethical review required for the protection of human subjects in compliance with the NIH Policy on the Use of a sIRB for Multi-Site Research. The Mass General Brigham (MGB) IRB will serve as the sIRB of record for this multi-center study and will facilitate efficient, effective, and consistent review of the proposed research. The MGB IRB provides ethical and scientific review and continuing oversight for the human subjects research at MGH and BWH.

10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

- External site monitoring visits will be performed remotely or in-person by the DCC monitors.
- On-site or remote monitoring will occur prior to the initiation of the site, after the first participant is enrolled, and yearly thereafter (and more frequently as needed) to review regulatory documents and monitor medical records against the study database. More frequent on-site or remote visits will occur if there is a problem at the site or a new satellite or subcontracted site is added. Emphasis will be placed on the process used to consent participants, compliance with regulatory requirements and the study protocol, study drug handling, and values of key endpoints.
- Records of IRB approvals and subjects' charts will be examined on a spot check basis by study monitors.
- Source documents will be reviewed for targeted source data verification related to study endpoints, safety and other key data variables.
- Site PIs will be provided copies of monitoring reports within 14 business days of the monitoring visit.
- Each clinical trial site will provide direct access to all source data/documents and reports for the purpose of monitoring and auditing by the DCC on behalf of the IND Sponsor, and inspection by local and regulatory authorities.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. Each site will operate under a comprehensive Quality Management Plan, which incorporates internal Quality Control (QC) activities prior to data entry and regular internal Quality Assurance (QA) review of subjects' charts.

QC procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Electronic source data are data initially recorded in electronic form.

Study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data should be entered in the electronic case report form (eCRF) in StudyTRAX as directed on the source document worksheets. Data recorded in the eCRF derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into StudyTRAX, a 21 CFR Part 11-compliant data capture system provided by the DCC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.
The study Manual of Operations will provide additional detail on data collection procedures, including source documentation, eCRF completion guidelines, data handling procedures and procedures for data monitoring and quality control that ensure these data are accurate, consistent, complete and reliable and in accordance with ICH 36.

10.1.9.2 STUDY RECORDS RETENTION

Study documents should be retained for at least seven years after study completion. No records will be destroyed without the written consent of the IND Sponsor. It is the responsibility of the IND Sponsor to inform the site investigators when these documents no longer need to be retained.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations in a timely manner. All deviations must be addressed in study source documents and will be reported to the DCC. Major protocol deviations, such as enrollment violations or any deviation that involves risks to participants or others, will be reported to the DCC within 4 business days of awareness. The CCC Project Manager will be responsible for reporting these to the single IRB within 5 business days. Minor deviations will be reported to the DCC as part of the site monitoring visits.

Sites should also follow their local IRB's policy on reporting protocol deviations for studies relying on a single IRB. The site investigator is responsible for knowing and adhering to the sIRB and local site IRB requirements.

10.1.11 PUBLICATION AND DATA SHARING POLICY

Results from this study will be reported according to guidelines established by the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. The final peer-reviewed journal manuscripts will be submitted to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. All biomarker data generated in this study will be shared with the scientific community following the appropriate guidelines for data deposition.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by any outside party, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. All persons involved in study are expected to take the appropriate actions to ensure that they are in compliance with the financial disclosure requirements of their respective institutions.

10.2 ABBREVIATIONS

ABG	Arterial Blood Gas
AE	Adverse Event
ALI	Acute Lung Injury
ARDS	Acute Respiratory Distress Syndrome
BAL	Bronchoalveolar Lavage
BUN	Blood Urea Nitrogen
BWH	Brigham and Women's Hospital
CFK equation	Coburn-Foster-Kane equation
CFR	Code of Federal Regulations
CHF	Congestive Heart Failure
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
CO	Carbon Monoxide
COHb	Carboxyhemoglobin
CONSORT	Consolidated Standards of Reporting Trials
CPAP	Continuous Positive Airway Pressure

CRF	Case Report Form
DBP	Diastolic Blood Pressure
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DLCO	Diffusing capacity of the Lung for Carbon Monoxide
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Forms
EKG	Electrocardiogram
EC	Executive Committee
FDA	Food and Drug Administration
FiO2	Fraction of Inspired Oxygen
GCP	Good Clinical Practice
GCS	Glasgow Coma Scale
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
iCO	Inhaled Carbon Monoxide
ICU	Intensive Care Unit
IDE	Investigational Device Exemption
IMV	Intermittent Mechanical Ventilation
IND	Investigational New Drug Application
INR	International Normalized Ratio
IRB	Institutional Review Board
ITT	Intention-To-Treat
LAR	Legally Authorized Representative
LIS	Lung Injury Score
LM	Lipid Mediator
MedDRA	Medical Dictionary for Regulatory Activities
mBW	Measured Body Weight
mtDNA	Mitochondrial DNA
MGH	Massachusetts General Hospital
MOP	Manual of Procedures
NCT	National Clinical Trial
NIH	National Institutes of Health
NO	Nitric Oxide
NYHA	New York Heart Association
OR	Odds Ratio
ORP	Office of Research Protections
$PaCO_2$	Partial pressure of arterial carbon dioxide
PaO_2	Partial pressure of arterial oxygen
PBMC	Peripheral Blood Mononuclear Cell
PEEP	Positive End-Expiratory Pressure
PI	Principal Investigator
Pplat	Plateau pressure
ppm	Parts per million
PS	Pressure Support Ventilation
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan

SBP	Systolic Blood Pressure
SBT	Spontaneous Breathing Trial
sIRB	Single Institutional Review Board
SOA	Schedule of Activities
SOC	System Organ Class
SOFA	Sequential Organ Failure Assessment
SOP	Standard Operating Procedure
SpCO	Non-invasive COHb by pulse oximetry
SPM	Specialized Pro-resolving Mediator
UAB	Unassisted Breathing
UP	Unanticipated Problem
US	United States
VBG	Venous Blood Gas
VFD	Ventilator-free Days
V_A	Alveolar ventilation
Vd/Vt	Dead space
vWF	von Willebrand factor
WBC	White Blood Cell

10.3 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
2.0	5/31/2022	**Synopsis:** Two additional sites (Washington University, and New York-Presbyterian Brooklyn Methodist Hospital have been added to the protocol.	Washington University and New York-Presbyterian Brooklyn Methodist Hospital will be participating as sites in the trial.
2.0	5/31/2022	**6.1.8, 8.7.3:** Clarification of criteria used to define acute MI and acute stroke.	The DSMB requested clarification of how these administration-related AEs would be defined.
2.0	5/31/2022	**6.3, 8.3.1:** The language regarding stratification by site was revised.	An error was noted in the protocol. The randomization is not stratified by site or any other factor
2.0	5/31/2022	**Cover, 6.3, 10.1.5:** The PI was changed from Dr. Laura Fredenburgh to Dr. Rebecca Baron. Dr. Baron was removed as Medical Monitor.	Dr. Rebecca Baron is the new PI of the study and will no longer serve as Medical Monitor. Dr. Taylor Thompson and Dr. Crystal North will serve as Medical Monitors.
2.0	5/31/2022	**8.7.5:** Clarification of who will collect AE information and assess their relationship to the study drug.	The DSMB requested clarification of who would be monitoring AEs and their relationship to the study team.
2.0	5/31/2022	**8.7.7:** Clarification that investigators will report AEs by entering the event information into the Studytrax database.	StudyTrax will automatically send an email alert to the CCC and DCC study leadership.
2.0	5/31/2022	**9.2, 9.4.2, 9.4.5:** Revision of the sample size determination section to clarify the power calculations. Revision of language and Table 6 regarding the power to detect AEs based on the true AE rate and sample size.	The prior language was incorrect and an error was noted in the probability calculations.
2.0	5/31/2022	**9.4.3, 9.4.4:** Revision of language, Tables 7 and 8 regarding the detectable differences in secondary and exploratory endpoints with our sample size.	An error was noted in these calculations.
2.0	5/31/2022	**9.4.6, 10.1.6:** Revision of the DSMB meeting frequency and SAE reporting to the DSMB.	The DSMB requests meeting twice per year or after 12 subjects are enrolled, whichever occurs sooner, to review the safety data in an unblinded fashion. All related SAEs will be reported to the DSMB in an expedited fashion and the DSMB would have the option after every SAE report to request an *ad hoc* meeting if safety concerns arise.

11 REFERENCES

1. Liu KD, Wilson JG, Zhuo H, et al. Design and implementation of the start (stem cells for ards treatment) trial, a phase 1/2 trial of human mesenchymal stem/stromal cells for the treatment of moderate-severe acute respiratory distress syndrome. Annals of intensive care 2014;4:22.

2. Wilson JG, Liu KD, Zhuo H, et al. Mesenchymal stem (stromal) cells for treatment of ards: A phase 1 clinical trial. The Lancet Respiratory medicine 2015;3:24-32.

3. Ranieri VM, Rubenfeld GD, Thompson BT, et al. Acute respiratory distress syndrome: The berlin definition. Jama 2012;307:2526-33.

4. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). Jama 2016;315:801-10.

5. Brealey D, Brand M, Hargreaves I, et al. Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet 2002;360:219-23.

6. Carre JE, Orban JC, Re L, et al. Survival in critical illness is associated with early activation of mitochondrial biogenesis. American journal of respiratory and critical care medicine 2010;182:745-51.

7. Nakahira K, Kyung SY, Rogers AJ, et al. Circulating mitochondrial DNA in patients in the icu as a marker of mortality: Derivation and validation. PLoS medicine 2013;10:e1001577; discussion e.

8. Harrington JS, Choi AMK, Nakahira K. Mitochondrial DNA in sepsis. Current opinion in critical care 2017;23:284-90.

9. Jung SS, Moon JS, Xu JF, et al. Carbon monoxide negatively regulates nlrp3 inflammasome activation in macrophages. Am J Physiol Lung Cell Mol Physiol 2015;308:L1058-67.

10. Rhodes MA, Carraway MS, Piantadosi CA, et al. Carbon monoxide, skeletal muscle oxidative stress, and mitochondrial biogenesis in humans. Am J Physiol Heart Circ Physiol 2009;297:H392-9.

11. Rubenfeld GD, Caldwell E, Peabody E, et al. Incidence and outcomes of acute lung injury. N Engl J Med 2005;353:1685-93.

12. Ware LB, Matthay MA. The acute respiratory distress syndrome. N Engl J Med 2000;342:1334-49.

13. The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. N Engl J Med 2000;342:1301-8.

14. Briel M, Meade M, Mercat A, et al. Higher vs lower positive end-expiratory pressure in patients with acute lung injury and acute respiratory distress syndrome: Systematic review and meta-analysis. Jama 2010;303:865-73.

15. Guerin C, Reignier J, Richard JC, et al. Prone positioning in severe acute respiratory distress syndrome. N Engl J Med 2013;368:2159-68.

16. Papazian L, Forel JM, Gacouin A, et al. Neuromuscular blockers in early acute respiratory distress syndrome. N Engl J Med 2010;363:1107-16.

17. Belenkiy SM, Buel AR, Cannon JW, et al. Acute respiratory distress syndrome in wartime military burns: Application of the berlin criteria. The journal of trauma and acute care surgery 2014;76:821-7.

18. Bellani G, Laffey JG, Pham T, et al. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. Jama 2016;315:788-800.

19. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the united states: Analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001;29:1303-10.

20. Angus DC, Wax RS. Epidemiology of sepsis: An update. Crit Care Med 2001;29:S109-16.

21. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003;348:138-50.

22. Morse D, Choi AM. Heme oxygenase-1: From bench to bedside. American journal of respiratory and critical care medicine 2005;172:660-70.

23. Otterbein LE, Choi AM. Heme oxygenase: Colors of defense against cellular stress. American journal of physiology Lung cellular and molecular physiology 2000;279:L1029-37.

24. Otterbein LE, Mantell LL, Choi AM. Carbon monoxide provides protection against hyperoxic lung injury. Am J Physiol 1999;276:L688-94.

25. Otterbein LE, Otterbein SL, Ifedigbo E, et al. Mkk3 mitogen-activated protein kinase pathway mediates carbon monoxide-induced protection against oxidant-induced lung injury. Am J Pathol 2003;163:2555-63.

26. Otterbein LE, Bach FH, Alam J, et al. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. Nat Med 2000;6:422-8.

27. Brouard S, Otterbein LE, Anrather J, et al. Carbon monoxide generated by heme oxygenase 1 suppresses endothelial cell apoptosis. J Exp Med 2000;192:1015-26.

28. Zhang X, Shan P, Alam J, Davis RJ, Flavell RA, Lee PJ. Carbon monoxide modulates fas/fas ligand, caspases, and bcl-2 family proteins via the p38alpha mitogen-activated protein kinase pathway during ischemia-reperfusion lung injury. J Biol Chem 2003;278:22061-70.

29. Song R, Mahidhara RS, Liu F, Ning W, Otterbein LE, Choi AM. Carbon monoxide inhibits human airway smooth muscle cell proliferation via mitogen-activated protein kinase pathway. Am J Respir Cell Mol Biol 2002;27:603-10.

30. Zhou Z, Song R, Fattman CL, et al. Carbon monoxide suppresses bleomycin-induced lung fibrosis. Am J Pathol 2005;166:27-37.

31. Fujita T, Toda K, Karimova A, et al. Paradoxical rescue from ischemic lung injury by inhaled carbon monoxide driven by derepression of fibrinolysis. Nat Med 2001;7:598-604.

32. Chung SW, Liu X, Macias AA, Baron RM, Perrella MA. Heme oxygenase-1-derived carbon monoxide enhances the host defense response to microbial sepsis in mice. J Clin Invest 2008;118:239-47.

33. Lee S, Lee SJ, Coronata AA, et al. Carbon monoxide confers protection in sepsis by enhancing beclin 1-dependent autophagy and phagocytosis. Antioxid Redox Signal 2014;20:432-42.

34. Chiang N, Shinohara M, Dalli J, et al. Inhaled carbon monoxide accelerates resolution of inflammation via unique proresolving mediator-heme oxygenase-1 circuits. J Immunol 2013;190:6378-88.

35. Pecorella SR, Potter JV, Cherry AD, et al. The ho-1/co system regulates mitochondrial-capillary density relationships in human skeletal muscle. Am J Physiol Lung Cell Mol Physiol 2015;309:L857-71.

36. Piantadosi CA, Withers CM, Bartz RR, et al. Heme oxygenase-1 couples activation of mitochondrial biogenesis to anti-inflammatory cytokine expression. J Biol Chem 2011;286:16374-85.

37. MacGarvey NC, Suliman HB, Bartz RR, et al. Activation of mitochondrial biogenesis by heme oxygenase-1-mediated nf-e2-related factor-2 induction rescues mice from lethal staphylococcus aureus sepsis. American journal of respiratory and critical care medicine 2012;185:851-61.

38. Fredenburgh LE, Kraft BD, Hess DR, et al. Effects of inhaled co administration on acute lung injury in baboons with pneumococcal pneumonia. Am J Physiol Lung Cell Mol Physiol 2015;309:L834-46.

39. Lee SJ, Ryter SW, Xu JF, et al. Carbon monoxide activates autophagy via mitochondrial reactive oxygen species formation. Am J Respir Cell Mol Biol 2011;45:867-73.

40. Ghanta S, Tsoyi K, Liu X, et al. Mesenchymal stromal cells deficient in autophagy proteins are susceptible to oxidative injury and mitochondrial dysfunction. Am J Respir Cell Mol Biol 2017;56:300-9.

41. Dalli J, Kraft BD, Colas RA, et al. The regulation of proresolving lipid mediator profiles in baboon pneumonia by inhaled carbon monoxide. Am J Respir Cell Mol Biol 2015;53:314-25.

42. Shinohara M, Kibi M, Riley IR, et al. Cell-cell interactions and bronchoconstrictor eicosanoid reduction with inhaled carbon monoxide and resolvin d1. American journal of physiology Lung cellular and molecular physiology 2014;307:L746-57.

43. Tsoyi K, Hall SR, Dalli J, et al. Carbon monoxide improves efficacy of mesenchymal stromal cells during sepsis by production of specialized proresolving lipid mediators. Crit Care Med 2016;44:e1236-e45.

44. Ryter SW, Alam J, Choi AM. Heme oxygenase-1/carbon monoxide: From basic science to therapeutic applications. Physiol Rev 2006;86:583-650.

45. Dolinay T, Szilasi M, Liu M, Choi AM. Inhaled carbon monoxide confers antiinflammatory effects against ventilator-induced lung injury. Am J Respir Crit Care Med 2004;170:613-20.

46. Hoetzel A, Dolinay T, Vallbracht S, et al. Carbon monoxide protects against ventilator-induced lung injury via ppar-gamma and inhibition of egr-1. Am J Respir Crit Care Med 2008;177:1223-32.

47. Hoetzel A, Schmidt R, Vallbracht S, et al. Carbon monoxide prevents ventilator-induced lung injury via caveolin-1. Crit Care Med 2009;37:1708-15.

48. Kohmoto J, Nakao A, Kaizu T, et al. Low-dose carbon monoxide inhalation prevents ischemia/reperfusion injury of transplanted rat lung grafts. Surgery 2006;140:179-85.

49. Kohmoto J, Nakao A, Stolz DB, et al. Carbon monoxide protects rat lung transplants from ischemia-reperfusion injury via a mechanism involving p38 mapk pathway. Am J Transplant 2007;7:2279-90.

50. Zhang X, Shan P, Otterbein LE, et al. Carbon monoxide inhibition of apoptosis during ischemia-reperfusion lung injury is dependent on the p38 mitogen-activated protein kinase pathway and involves caspase 3. J Biol Chem 2003;278:1248-58.

51. Morse D, Pischke SE, Zhou Z, et al. Suppression of inflammatory cytokine production by carbon monoxide involves the jnk pathway and ap-1. J Biol Chem 2003;278:36993-8.

52. Sarady JK, Zuckerbraun BS, Bilban M, et al. Carbon monoxide protection against endotoxic shock involves reciprocal effects on inos in the lung and liver. Faseb J 2004;18:854-6.

53. Zuckerbraun BS, McCloskey CA, Gallo D, et al. Carbon monoxide prevents multiple organ injury in a model of hemorrhagic shock and resuscitation. Shock 2005;23:527-32.

54. Mitchell LA, Channell MM, Royer CM, Ryter SW, Choi AM, McDonald JD. Evaluation of inhaled carbon monoxide as an anti-inflammatory therapy in a nonhuman primate model of lung inflammation. Am J Physiol Lung Cell Mol Physiol 2010;299:L891-7.

55. Nakahira K, Choi AM. Carbon monoxide in the treatment of sepsis. American journal of physiology Lung cellular and molecular physiology 2015;309:L1387-93.

56. Kraft BD, Piantadosi CA, Benjamin AM, et al. Development of a novel preclinical model of pneumococcal pneumonia in nonhuman primates. Am J Respir Cell Mol Biol 2014;50:995-1004.

57. Zuckerbraun BS, Chin BY, Wegiel B, et al. Carbon monoxide reverses established pulmonary hypertension. J Exp Med 2006;203:2109-19.

58. Christou H, Morita T, Hsieh CM, et al. Prevention of hypoxia-induced pulmonary hypertension by enhancement of endogenous heme oxygenase-1 in the rat. Circ Res 2000;86:1224-9.

59. Minamino T, Christou H, Hsieh CM, et al. Targeted expression of heme oxygenase-1 prevents the pulmonary inflammatory and vascular responses to hypoxia. Proc Natl Acad Sci U S A 2001;98:8798-803.

60. Yet SF, Perrella MA, Layne MD, et al. Hypoxia induces severe right ventricular dilatation and infarction in heme oxygenase-1 null mice. J Clin Invest 1999;103:R23-9.

61. Kim HP, Wang X, Nakao A, et al. Caveolin-1 expression by means of p38beta mitogen-activated protein kinase mediates the antiproliferative effect of carbon monoxide. Proc Natl Acad Sci U S A 2005;102:11319-24.

62. Otterbein LE, Zuckerbraun BS, Haga M, et al. Carbon monoxide suppresses arteriosclerotic lesions associated with chronic graft rejection and with balloon injury. Nat Med 2003;9:183-90.

63. Raman KG, Barbato JE, Ifedigbo E, et al. Inhaled carbon monoxide inhibits intimal hyperplasia and provides added benefit with nitric oxide. J Vasc Surg 2006;44:151-8.

64. Ramlawi B, Scott JR, Feng J, et al. Inhaled carbon monoxide prevents graft-induced intimal hyperplasia in swine. J Surg Res 2007;138:121-7.

65. True AL, Olive M, Boehm M, et al. Heme oxygenase-1 deficiency accelerates formation of arterial thrombosis through oxidative damage to the endothelium, which is rescued by inhaled carbon monoxide. Circ Res 2007;101:893-901.

66. Akamatsu Y, Haga M, Tyagi S, et al. Heme oxygenase-1-derived carbon monoxide protects hearts from transplant associated ischemia reperfusion injury. FASEB J 2004;18:771-2.

67. Faleo G, Neto JS, Kohmoto J, et al. Carbon monoxide ameliorates renal cold ischemia-reperfusion injury with an upregulation of vascular endothelial growth factor by activation of hypoxia-inducible factor. Transplantation 2008;85:1833-40.

68. Kaizu T, Ikeda A, Nakao A, et al. Protection of transplant-induced hepatic ischemia/reperfusion injury with carbon monoxide via mek/erk1/2 pathway downregulation. Am J Physiol Gastrointest Liver Physiol 2008;294:G236-44.

69. Nakao A, Faleo G, Nalesnik MA, Seda-Neto J, Kohmoto J, Murase N. Low-dose carbon monoxide inhibits progressive chronic allograft nephropathy and restores renal allograft function. Am J Physiol Renal Physiol 2009;297:F19-26.

70. Nakao A, Kimizuka K, Stolz DB, et al. Carbon monoxide inhalation protects rat intestinal grafts from ischemia/reperfusion injury. Am J Pathol 2003;163:1587-98.

71. Nakao A, Toyokawa H, Abe M, et al. Heart allograft protection with low-dose carbon monoxide inhalation: Effects on inflammatory mediators and alloreactive t-cell responses. Transplantation 2006;81:220-30.

72. Neto JS, Nakao A, Kimizuka K, et al. Protection of transplant-induced renal ischemia-reperfusion injury with carbon monoxide. Am J Physiol Renal Physiol 2004;287:F979-89.

73. Sato K, Balla J, Otterbein L, et al. Carbon monoxide generated by heme oxygenase-1 suppresses the rejection of mouse-to-rat cardiac transplants. J Immunol 2001;166:4185-94.

74. Song R, Kubo M, Morse D, et al. Carbon monoxide induces cytoprotection in rat orthotopic lung transplantation via anti-inflammatory and anti-apoptotic effects. Am J Pathol 2003;163:231-42.

75. Boutros C, Zegdi R, Lila N, et al. Carbon monoxide can prevent acute lung injury observed after ischemia reperfusion of the lower extremities. J Surg Res 2007;143:437-42.

76. Mishra S, Fujita T, Lama VN, et al. Carbon monoxide rescues ischemic lungs by interrupting mapk-driven expression of early growth response 1 gene and its downstream target genes. Proc Natl Acad Sci U S A 2006;103:5191-6.

77. Scott JR, Cukiernik MA, Ott MC, et al. Low-dose inhaled carbon monoxide attenuates the remote intestinal inflammatory response elicited by hindlimb ischemia-reperfusion. Am J Physiol Gastrointest Liver Physiol 2009;296:G9-G14.

78. Nakao A, Moore BA, Murase N, et al. Immunomodulatory effects of inhaled carbon monoxide on rat syngeneic small bowel graft motility. Gut 2003;52:1278-85.

79. Goebel U, Mecklenburg A, Siepe M, et al. Protective effects of inhaled carbon monoxide in pig lungs during cardiopulmonary bypass are mediated via an induction of the heat shock response. Br J Anaesth 2009;103:173-84.

80. Goebel U, Siepe M, Mecklenburg A, et al. Reduced pulmonary inflammatory response during cardiopulmonary bypass: Effects of combined pulmonary perfusion and carbon monoxide inhalation. Eur J Cardiothorac Surg 2008;34:1165-72.

81. Goebel U, Siepe M, Mecklenburg A, et al. Carbon monoxide inhalation reduces pulmonary inflammatory response during cardiopulmonary bypass in pigs. Anesthesiology 2008;108:1025-36.

82. Lavitrano M, Smolenski RT, Musumeci A, et al. Carbon monoxide improves cardiac energetics and safeguards the heart during reperfusion after cardiopulmonary bypass in pigs. FASEB J 2004;18:1093-5.

83. Piantadosi CA, Carraway MS, Babiker A, Suliman HB. Heme oxygenase-1 regulates cardiac mitochondrial biogenesis via nrf2-mediated transcriptional control of nuclear respiratory factor-1. Circ Res 2008;103:1232-40.

84. Suliman HB, Carraway MS, Ali AS, Reynolds CM, Welty-Wolf KE, Piantadosi CA. The co/ho system reverses inhibition of mitochondrial biogenesis and prevents murine doxorubicin cardiomyopathy. J Clin Invest 2007;117:3730-41.

85. Ameredes BT, Otterbein LE, Kohut LK, Gligonic AL, Calhoun WJ, Choi AM. Low-dose carbon monoxide reduces airway hyperresponsiveness in mice. American journal of physiology Lung cellular and molecular physiology 2003;285:L1270-6.

86. Chapman JT, Otterbein LE, Elias JA, Choi AM. Carbon monoxide attenuates aeroallergen-induced inflammation in mice. American journal of physiology Lung cellular and molecular physiology 2001;281:L209-16.

87. Pamplona A, Ferreira A, Balla J, et al. Heme oxygenase-1 and carbon monoxide suppress the pathogenesis of experimental cerebral malaria. Nat Med 2007;13:703-10.

88. Tsui TY, Obed A, Siu YT, et al. Carbon monoxide inhalation rescues mice from fulminant hepatitis through improving hepatic energy metabolism. Shock 2007;27:165-71.

89. Zuckerbraun BS, Billiar TR, Otterbein SL, et al. Carbon monoxide protects against liver failure through nitric oxide-induced heme oxygenase 1. J Exp Med 2003;198:1707-16.

90. Hegazi RA, Rao KN, Mayle A, Sepulveda AR, Otterbein LE, Plevy SE. Carbon monoxide ameliorates chronic murine colitis through a heme oxygenase 1-dependent pathway. J Exp Med 2005;202:1703-13.

91. Moore BA, Overhaus M, Whitcomb J, et al. Brief inhalation of low-dose carbon monoxide protects rodents and swine from postoperative ileus. Crit Care Med 2005;33:1317-26.

92. Zuckerbraun BS, Otterbein LE, Boyle P, et al. Carbon monoxide protects against the development of experimental necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 2005;289:G607-13.

93. Beckman JD, Belcher JD, Vineyard JV, et al. Inhaled carbon monoxide reduces leukocytosis in a murine model of sickle cell disease. Am J Physiol Heart Circ Physiol 2009;297:H1243-53.

94. Takagi T, Naito Y, Inoue M, et al. Inhalation of carbon monoxide ameliorates collagen-induced arthritis in mice and regulates the articular expression of il-1beta and mcp-1. Inflammation 2009;32:83-8.

95. Wang L, Lee JY, Kwak JH, He Y, Kim SI, Choi ME. Protective effects of low-dose carbon monoxide against renal fibrosis induced by unilateral ureteral obstruction. Am J Physiol Renal Physiol 2008;294:F508-17.

96. Coburn RF, Forster RE, Kane PB. Considerations of the physiological variables that determine the blood carboxyhemoglobin concentration in man. J Clin Invest 1965;44:1899-910.

97. Bernard TE, Duker J. Modeling carbon monoxide uptake during work. Am Ind Hyg Assoc J 1981;42:361-4.

98. Hauck H, Neuberger M. Carbon monoxide uptake and the resulting carboxyhemoglobin in man. Eur J Appl Physiol Occup Physiol 1984;53:186-90.

99. Joumard R, Chiron M, Vidon R, Maurin M, Rouzioux JM. Mathematical models of the uptake of carbon monoxide on hemoglobin at low carbon monoxide levels. Environ Health Perspect 1981;41:277-89.

100. Peterson JE, Stewart RD. Absorption and elimination of carbon monoxide by inactive young men. Arch Environ Health 1970;21:165-71.

101. Peterson JE, Stewart RD. Predicting the carboxyhemoglobin levels resulting from carbon monoxide exposures. J Appl Physiol 1975;39:633-8.

102. Tikuisis P, Buick F, Kane DM. Percent carboxyhemoglobin in resting humans exposed repeatedly to 1,500 and 7,500 ppm co. J Appl Physiol (1985) 1987;63:820-7.

103. Tikuisis P, Kane DM, McLellan TM, Buick F, Fairburn SM. Rate of formation of carboxyhemoglobin in exercising humans exposed to carbon monoxide. J Appl Physiol 1992;72:1311-9.

104. Hausberg M, Somers VK. Neural circulatory responses to carbon monoxide in healthy humans. Hypertension 1997;29:1114-8.

105. Mayr FB, Spiel A, Leitner J, et al. Effects of carbon monoxide inhalation during experimental endotoxemia in humans. Am J Respir Crit Care Med 2005;171:354-60.

106. Stewart RD, Peterson JE, Baretta ED, Bachand RT, Hosko MJ, Herrmann AA. Experimental human exposure to carbon monoxide. Arch Environ Health 1970;21:154-64.

107. Zevin S, Saunders S, Gourlay SG, Jacob P, Benowitz NL. Cardiovascular effects of carbon monoxide and cigarette smoking. J Am Coll Cardiol 2001;38:1633-8.

108. Ren X, Dorrington KL, Robbins PA. Respiratory control in humans after 8 h of lowered arterial po2, hemodilution, or carboxyhemoglobinemia. J Appl Physiol 2001;90:1189-95.

109. Bathoorn E, Slebos DJ, Postma DS, et al. Anti-inflammatory effects of inhaled carbon monoxide in patients with copd: A pilot study. Eur Respir J 2007;30:1131-7.

110. Rosas IO, Goldberg HJ, Collard HR, et al. A phase ii clinical trial of low-dose inhaled carbon monoxide in idiopathic pulmonary fibrosis. Chest 2018;153:94-104.

111. Fredenburgh LE, Perrella MA, Barragan-Bradford D, et al. A phase i trial of low dose inhaled carbon monoxide for the treatment of sepsis-induced ards. American journal of respiratory and critical care medicine 2018;197:A1125.

112. Fredenburgh LE, Perrella MA, Barragan-Bradford D, et al. A phase i trial of low-dose inhaled carbon monoxide in sepsis-induced ards. JCI insight 2018;3.

113. Behera D, Dash S, Dinakar M. Blood carboxyhaemoglobin levels in indian bidi and cigarette smokers. Respiration 1991;58:26-8.

114. Behera D, Dash S, Dinakar M. Correlation of smoking behaviour and blood carboxyhaemoglobin in bidi and cigarette smokers. Indian J Chest Dis Allied Sci 1991;33:43-6.

115. Stewart RD, Hake CL, Wu A, Stewart TA, Kalbfleisch JH. Carboxyhemoglobin trend in chicago blood donors, 1970-1974. Arch Environ Health 1976;31:280-5.

116. Rooney KT, Goldberg HJ, Hunninghake GM, et al. The effect of inhaled low dose carbon monoxide on cytokine/chemokine production in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2016;193 A4542.

117. Clausen JL ZL. Pulmonary function testing, guidelines and controversies: Equipment, methods, and normal values, . New York: Academic Press 1982.

118. Gong MN, Bajwa EK, Thompson BT, Christiani DC. Body mass index is associated with the development of acute respiratory distress syndrome. Thorax 2010;65:44-50.

119. Kallet RH, Alonso JA, Pittet JF, Matthay MA. Prognostic value of the pulmonary dead-space fraction during the first 6 days of acute respiratory distress syndrome. Respir Care 2004;49:1008-14.

120. Kallet RH, Zhuo H, Liu KD, et al. The association between physiologic dead-space fraction and mortality in subjects with ards enrolled in a prospective multi-center clinical trial. Respir Care 2014;59:1611-8.

121. Macnaughton PD, Evans TW. Measurement of lung volume and dlco in acute respiratory failure. American journal of respiratory and critical care medicine 1994;150:770-5.

122. Nuckton TJ, Alonso JA, Kallet RH, et al. Pulmonary dead-space fraction as a risk factor for death in the acute respiratory distress syndrome. N Engl J Med 2002;346:1281-6.

123. Siddiki H, Kojicic M, Li G, et al. Bedside quantification of dead-space fraction using routine clinical data in patients with acute lung injury: Secondary analysis of two prospective trials. Crit Care 2010;14:R141.

124. Sheridan RL, Hess D. Inhaled nitric oxide in inhalation injury. J Burn Care Res 2009;30:162-4.

125. Gomez H, Kautza B, Escobar D, et al. Inhaled carbon monoxide protects against the development of shock and mitochondrial injury following hemorrhage and resuscitation. PloS one 2015;10:e0135032.

126. Kawanishi S, Takahashi T, Morimatsu H, et al. Inhalation of carbon monoxide following resuscitation ameliorates hemorrhagic shock-induced lung injury. Molecular medicine reports 2013;7:3-10.

127. Kanagawa F, Takahashi T, Inoue K, et al. Protective effect of carbon monoxide inhalation on lung injury after hemorrhagic shock/resuscitation in rats. The Journal of trauma 2010;69:185-94.

128. Choi YK, Maki T, Mandeville ET, et al. Dual effects of carbon monoxide on pericytes and neurogenesis in traumatic brain injury. Nat Med 2016.

129. O'Donnell RD, Mikulka P, Heinig P, Theodore J. Low level carbon monoxide exposure and human psychomotor performance. Toxicology and applied pharmacology 1971;18:593-602.

130. Piantadosi CA, Suliman HB. Redox regulation of mitochondrial biogenesis. Free radical biology & medicine 2012;53:2043-53.

131. Mizumura K, Cloonan S, Choi ME, et al. Autophagy: Friend or foe in lung disease? Annals of the American Thoracic Society 2016;13 Suppl 1:S40-7.

132. Nakahira K, Pabon Porras MA, Choi AM. Autophagy in pulmonary diseases. American journal of respiratory and critical care medicine 2016;194:1196-207.

133. Ryter SW, Choi AM. Targeting heme oxygenase-1 and carbon monoxide for therapeutic modulation of inflammation. Translational research : the journal of laboratory and clinical medicine 2016;167:7-34.

134. Clark CJ, Campbell D, Reid WH. Blood carboxyhaemoglobin and cyanide levels in fire survivors. Lancet 1981;1:1332-5.

135. Jay GD, McKindley DS. Alterations in pharmacokinetics of carboxyhemoglobin produced by oxygen under pressure. Undersea & hyperbaric medicine : journal of the Undersea and Hyperbaric Medical Society, Inc 1997;24:165-73.

136. Weaver LK, Howe S, Hopkins R, Chan KJ. Carboxyhemoglobin half-life in carbon monoxide-poisoned patients treated with 100% oxygen at atmospheric pressure. Chest 2000;117:801-8.

137. Hampson NB, Piantadosi CA, Thom SR, Weaver LK. Practice recommendations in the diagnosis, management, and prevention of carbon monoxide poisoning. American journal of respiratory and critical care medicine 2012;186:1095-101.

138. Lopez DM, Weingarten-Arams JS, Singer LP, Conway EE, Jr. Relationship between arterial, mixed venous, and internal jugular carboxyhemoglobin concentrations at low, medium, and high concentrations in a piglet model of carbon monoxide toxicity. Crit Care Med 2000;28:1998-2001.

139. Touger M, Gallagher EJ, Tyrell J. Relationship between venous and arterial carboxyhemoglobin levels in patients with suspected carbon monoxide poisoning. Annals of emergency medicine 1995;25:481-3.

140. Wagner GS, Macfarlane P, Wellens H, et al. Aha/accf/hrs recommendations for the standardization and interpretation of the electrocardiogram: Part vi: Acute ischemia/infarction: A scientific statement from the american heart association electrocardiography and arrhythmias committee, council on clinical cardiology; the american college of cardiology foundation; and the heart rhythm society. Endorsed by the international society for computerized electrocardiology. J Am Coll Cardiol 2009;53:1003-11.

141. American College of Emergency Physicians, Society for Cardiovascular Angiography Interventions, O'Gara PT, et al. 2013 accf/aha guideline for the management of st-elevation myocardial infarction: Executive summary: A report of the american college of cardiology foundation/american heart association task force on practice guidelines. J Am Coll Cardiol 2013;61:485-510.

142. O'Connor RE, Brady W, Brooks SC, et al. Part 10: Acute coronary syndromes: 2010 american heart association guidelines for cardiopulmonary resuscitation and emergency cardiovascular care. Circulation 2010;122:S787-817.

143. Rivara MB, Bajwa EK, Januzzi JL, Gong MN, Thompson BT, Christiani DC. Prognostic significance of elevated cardiac troponin-t levels in acute respiratory distress syndrome patients. PloS one 2012;7:e40515.

144. Thygesen K, Alpert JS, Jaffe AS, et al. Fourth universal definition of myocardial infarction (2018). J Am Coll Cardiol 2018;72:2231-64.

145. Sacco RL, Kasner SE, Broderick JP, et al. An updated definition of stroke for the 21st century: A statement for healthcare professionals from the american heart association/american stroke association. Stroke 2013;44:2064-89.

146. Pocock S. Clinical trials: A practical approach. New York: John Wiley; 1984.