Vitamin **C** Infusion for **TR**eatment **I**n **S**epsis Associated **A**cute **L**ung **I**njury

CITRIS-ALI

Protocol Version 9

Protocol Changes (Versions 2 – 9)

- **Version 2 Changes:** May, 2014 — Small edits made to the protocol including the removal of Venous Blood Gases for research, other small changes. Consent changed to reflect that blood specimens would be saved for a repository. A DSMB memo is was also submitted. Included the phrase: no evidence of left atrial hypertension in inclusion criteria.
- **Version 3 Changes:** May, 2014 — Minor changes made to the ventilator weaning and fluid management protocol.
- **Version 4 Changes:** July, 2014 — The title of the study was changed from "sepsis-induced ARDS" to "sepsis-associated ARDS," and these changes were made throughout the protocol and consent. Body mass index over 40 was removed as part of the exclusion criteria.
- **Version 5 Changes:** November, 2014 — DSMB report submitted. Changes made to the protocol regarding specimen and data time points for clarification. Changes made to the inclusion exclusion criteria based on the suggestion of the DSMB and investigators. Changes were made to more clearly define patient population. More than seven days since starting mechanical ventilation removed from exclusion criteria. This is due to the fact that a patient can have new onset of ARDS not related to their time spent on a ventilator. With this amendment, now allowing for up to 2 liters of home oxygen therapy. This allows capture of patients with chronic obstructive lung disease. Still excluding interstitial lung disease (ILD) patients. ILD patients not on a ventilator added to exclusion criteria. Was in the exclusion criteria; however, now more visible now for clinical coordinators. Other minor changes. None of the changes affect safety or risk; nor do they require a change to the consent form.
- **Version 6 Changes:** March 2015 — Amendment served as our solution for monitoring blood glucose at the bedside. We made additional edits to the exclusion criteria. Excluding patients with no indwelling venous or arterial catheter in patients that require insulin in a manner that requires glucose being checked more than twice daily (e.g. continuous infusion, sliding scale). Updated the risk section in the consent form and protocol to reflect the risks associated with our modified glucose monitoring plan. Made minor administrative changes to the protocol.
- **Version 7 Changes:** December, 2015 — Administrative changes for clarity. Clarification that no bedside glucometer can be used for glucose monitoring while patients are in the CITRIS-ALI trial. Clarification on additional blood draw totals for glucose monitoring made.
- **Version 8 Changes:** July, 2016 — Dropped an enrollment site due to poor patient enrollment.
- Version 9 Changes: February, 2017 — Small administrative changes.

- The projected sample size for this study (n =170) should provide adequate power to detect an absolute 2 point difference on the average SOFA scores between the two study groups (13 vs. 11) with an average SD of 4.6. This will

provide an alpha level of 0.05 and a power of 80%.

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ABBREVIATIONS

ABG = Arterial blood gas
AKI = Acute Kidney Injury
ALI = Acute Lung Injury
Ang-2 = Angiopoietin-2
APACHE = Acute physiologic and chronic health evaluation
AscA = Ascorbic Acid (Vitamin C)
ARDS = Acute Respiratory Distress Syndrome
BIPAP = Bi-level Positive Airway Pressure
BMI = Body Mass Index
CCC = Clinical Coordinating Center
CK = Creatinine Kinase
CPAP = Continuous Positive Airway Pressure
CRP = C-reactive protein
Day 0 = Day of Randomizations
DHA = Dehydroascorbic Acid
DSMB = Data Safety Monitoring Board
FACTT = Fluid and Catheter Treatment Trial
FDA=Food and drug administration
FiO_2 = Fraction of Inspired Oxygen
FIP = Feces Induced Peritonitis
GCS = Glasgow Coma Scale
ICU = Intensive care Unit
IMV = Intermittent Mechanical Ventilation
IRB = Institutional Review Board
IVRS = Interactive Voice Response System
LPS = Lipopolysaccharide
MBW = measured body weight
NFκB= Nuclear factor kappa B
NHLBI = National Heart Lung and Blood Institute
NIV=Non-invasive ventilation
NOS= Nitric oxide synthase
OI = Oxygenation Index = [mean airway pressure x $Fi0_2$]/$Pa0_2$
$PaCO_2$= Partial pressure of arterial carbon dioxide
PaO_2 = Partial pressure of arterial oxygen
PBW = Predicted Body Weight
PCT = Procalcitonin
PCV = Pressure Control Ventilation
PEEP = Positive End-Expiratory Pressure
PIN = Personal Identification Number
Pplat = Plateau pressure
PSV = Pressure Support Ventilation
PAOP = Pulmonary Artery Occlusion Pressure
RAGE = receptor for advanced glycation end products
RCT = Randomized Controlled Trial
SBT = Spontaneous Breathing Trial
SIRS = Systemic Inflammatory Response Syndrome
SOFA =Sequential Organ Failure Assessment
SpO_2 = Oxygen Saturation
TFPI = Tissue Factor Pathway Inhibitor
TM = Thrombomodulin
VFD = Ventilator-free Day

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

Asian: A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.

Completing 48 hours of Unassisted Breathing (UAB) : Defined as the date (calendar day) that the subject reaches exactly 48 hours of UAB. Example: if subject meets UAB at 1900 on 6/1/14 and does not return to assisted breathing (AB), then the date of completing 48 hours of UAB would be 6/3/14.

Date of first UAB: Defined as the first day that the subject is on UAB from midnight to midnight. Example: if subject meets UAB at 1900 on 6/1/14, then the date of first UAB would be 6/2/14, as long as subject does not return to AB on 6/2/14.

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

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).

Severe Sepsis: SIRS criteria plus suspected or known infection plus organ dysfunction. Since intubation and hypoxemia is a requirement for enrollment into this trial, participants will, by definition, meet the SIRS criterion.

Study hospital: Defined as the hospital where the patient was randomized and 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 clinician should 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 collar (mask) breathing, or CPAP ≤ 5 without PSV or IMV assistance, or the use of noninvasive ventilation solely for sleep-disordered breathing. Assisted breathing is any level of ventilatory support at pressures higher that the unassisted breathing thresholds.

Part I: Study Summary

Title: Vitamin **C** **I**nfusion for **TR**eatment **I**n **S**epsis **Associated** **A**cute **L**ung **I**njury

(CITRIS-ALI)

Objective: To assess the efficacy of a 96-hour intravenous vitamin C infusion protocol (200 mg/kg per 24 hours) in patients with established acute lung injury (ALI) from sepsis. In this course of performing this phase II trial we will explore three hypotheses:

Hypothesis:

Hypothesis 1A: Vitamin C infusion will significantly attenuate sepsis associated systemic organ failure as measured by Sequential Organ Failure Assessment (SOFA) score

Hypothesis 1B: Vitamin C infusion will attenuate sepsis associated lung injury as assessed by oxygenation index and the VE 40 (see below)

Hypothesis 1C: Vitamin C infusion will attenuate biomarkers of inflammation (C-Reactive Protein, Procalcitonin), vascular injury (Thrombomodulin, Angiopoietin-2), alveolar epithelial injury (Receptor For Advanced Glycation End Products), while inducing the onset of a fibrinolytic state (Tissue Factor Pathway Inhibitor).

Study Design:

1. Multi-center, prospective, randomized, placebo-controlled clinical trial
2. A maximum of 170 patients will be enrolled
3. Participants will be randomized to receive either intravenous Vitamin C (mixed in 5% dextrose in water) or placebo (5% dextrose in water)
4. Active treatment will continue for 96 hours, discharge from study hospital, discharge from the ICU, study withdrawal, or death, whichever comes first.
5. All participants will be followed for a total of 60 days.

Analysis/Interim Monitoring:

1. The principal analysis will be on the basis of the intention-to-treat.
2. Protocol compliance will be monitored by the study team by presentation of 1st two enrolled subjects per site to the team. This will take place via investigator conference call and will address challenges encountered. Trial progress will be monitored by an independent Data and Safety Monitoring Board to determine if the study should stop for safety reasons. As an early phase study it is important to collect as much data as possible. For this reason the study will only be halted by the DSMB for reasons of patient safety concerns. The first scheduled analysis will occur after the enrollment of 80 patients or semi-annually, whichever happens first. The next review will occur after enrollment of the last enrolled subject, or semi-annually, whichever comes first. In the event that safety concerns arise prior to the scheduled analysis, the DSMB may request an unscheduled review at any time. The Data and Safety Monitoring Board (DSMB) will also monitor trial quality.
3. Regulatory compliance, GCP, and risk-based monitoring will be provided by an independent CRO.

CITRIS-ALI Inclusion and Exclusion Criteria:

The inclusion and exclusion criteria for the CITRIS-ALI trial is listed here and in the full CITRIS-ALI protocol. The definition of severe sepsis for this study is derived and defined as previously published in the referenced literature.[1,2,3]

CITRIS-ALI Inclusion Criteria:

Patients must have suspected or proven infection, and meet 2 out of 4 of the criteria for Systemic Inflammatory Response (SIRS) due to infection, and be accompanied by at least 1 criterion for sepsis associated organ dysfunction, *and* meet all 5 criteria for Acute Respiratory Distress Syndrome (ARDS).

1. *Suspected or proven infection:* (e.g., thorax, urinary tract, abdomen, skin, sinuses, central venous catheters, and central nervous system, see Appendix A).
2. *The presence of a systemic inflammatory response*: Defined as: *fever:* >38ºC (any route) or *hypothermia:* <36ºC (core temp only), *tachycardia:* heart rate > 90 beats/min or receiving medications that slow heart rate or paced rhythm, *leukocytosis*: >12,000 WBC/µL or *leukopenia*: <4,000 WBC/µL or >10% band forms. Respiratory rate > 20 breaths per minute or PaCO2 < 32 or invasive mechanical ventilation.
3. *The presence of sepsis associated organ dysfunction:* (any of the following thought to be due to infection)
    a. Sepsis associated hypotension (systolic blood pressure (SBP) < 90 mm Hg or an SBP decrease > 40 mm Hg unexplained by other causes or use of vasopressors for blood pressure support (epinephrine, norepinephrine, dopamine =/> 5mcg, phenylephrine)
    b. Arterial hypoxemia ($PaO_2/FiO_2 \leq 300$) or supplemental O2 > 6LPM.
    c. Lactate > upper limits of normal laboratory results
    d. Urine output < 0.5 ml/kg/hour for > two hours despite adequate fluid resuscitation
    e. Platelet count < 100,000 per mcL
    f. Coagulopathy (INR > 1.5)
    g. Bilirubin > 2 mg/dL
    h. Glasgow Coma Scale < 11 or a positive CAM ICU score
4. *ARDS characterized by all the following criteria*
    a. Lung injury of acute onset, within 1 week of an apparent clinical insult and with progression of respiratory symptoms
    b. Bilateral opacities on chest imaging not explained by other pulmonary pathology (e.g. pleural effusions, lung collapse, or nodules)
    c. Respiratory failure not explained by heart failure or volume overload
    d. Decreased arterial PaO2/FiO2 ratio ≤ 300 mm Hg
    e. Minimum PEEP of 5 cmH_2O

CITRIS-ALI Exclusion Criteria:

1. Known allergy to Vitamin C
2. inability to obtain consent;
3. age < 18 years;
4. Not on a ventilator
5. more than 48 hrs since meeting ARDS criteria;
6. No indwelling venous or arterial catheter in patients requiring insulin in a manner that requires glucose being checked more than twice daily (e.g. continuous infusion, sliding scale)
7. Presence of diabetic ketoacidosis
8. patient or surrogate or physician not committed to full support (not excluded if patient would receive all supportive care except for cardiac resuscitation);
9. pregnancy or breast feeding,
10. moribund patient not expected to survive 24 hours;
11. home mechanical ventilation (via tracheotomy or noninvasive) except for CPAP/BIPAP used only for sleep-disordered breathing;
12. on home O2 > 2LPM, except for with CPAP/BIPAP
13. diffuse alveolar hemorrhage (vasculitis);
14. interstitial lung disease requiring continuous home oxygen therapy;
15. Active kidney stone
16. Non English speaking;
17. ward of the state (inmate, other).

Primary Objective:

To assess the efficacy of a 96-hour high dose intravenous vitamin C infusion protocol (200 mg/kg per 24 hours) in patients with established ALI/ARDS that results from severe sepsis. Patients will be randomized to receive either: 1) ***Placebo*** (50 ml of 5% dextrose in water) or ***Vitamin C*** (sterile L-ascorbic acid for injection at 200 mg/kg per 24 hours with entire calculated 24 hour dose diluted in 200 ml of 5% dextrose in water). One fourth of the 24 hour calculated dosage will be administered in 30 minute intravenous infusions will occur every 6 hours.

Endpoints:

The CITRIS-ALI trial will depart from prior acute lung injury trials in that assessment of efficacy will not include 28-day all-cause mortality as a primary endpoint. As directed in **RFA-HL-12-022**, primary end points will focus on quantifiable measures of organ function and biomarker analysis (SOFA, CRP, Procalcitonin, Thrombomodulin). For this phase II trial we propose co-primary endpoints.

Primary Endpoint #1: Change in SOFA score at 96 hours as compared to baseline when compared to placebo.

Primary Endpoint #2: C-Reactive Protein and Thrombomodulin at study hours 0, 48, 96, 168 when compared to placebo.

Secondary Endpoints:

- Oxygenation Index (FiO_2 x Mean Airway Pressure/PO_2) at study hour 0, 48, 96, 168 if still intubated in ascorbate infused patient compared to placebo.
- VE-40 (Vent RR x TV/Weight) x ($PaCO_2$/40) at study hour 0, 48, 96, 168 if still intubated, in ascorbate infused patient compared to placebo
- SOFA scores at hours 48, 96, 168
- SOFA Score Components at hours 48,96, 168
    - PaO2/FiO2

    - SpO2/FiO2
    - Platelets
    - Total Bilirubin
    - Vasopressor status
    - GCS
    - Creatinine or Urine Output
- Procalcitonin, Receptor for Advanced Glycation End Products, Tissue Factor Pathway Inhibitor at study hour 0, 48, 96, 168
- Ascorbate level at hour 0, 48, 96, 168
- Ventilator Free Days to day 28
- ICU-free days at day 28
- All cause mortality to day 28
- Hospital-free days at day 60

Focused Safety Analysis: Given that L-Ascorbate is an "acid" the drug manufacturer adjusts the pH thus balancing pH at 7.4 and negating the acid effect of the drug. Unexplained metabolic acidosis will be addressed per standard of care for each participating institution.

Study Drug Dosing:

1. **First study drug dose** (L-ascorbic acid or placebo) will be considered "Dose 1" and will be administered within 6 hours of randomization or the earliest available time post any clinically indicated procedure which requires the patient to be off the unit. All doses will be administered in the ICU. Patients receiving vitamin C will receive 25% of the total daily calculated dosing (200mg/kg/24 hours) and will be infused over 30 minutes for this first dosing.
2. **Subsequent doses** which represent 25% of the day's total dose will be infused every six hours through 96 hours (+/- 3 hours).
    a. Timing of Dose 2 will be triggered by the physician order for q 6 hour administration and will therefore be listed on the bedside MAR. As such, timing of Dose 2 may be out of the +/- 3 hour window and will not trigger a protocol deviation.
    b. If for any reason any other maintenance dose is not administered within window, the dose will be skipped and the next scheduled dose will be given and documented in the data collection tool.

Drug level specimens (venous blood): Septic patients exhibit subnormal plasma ascorbate levels. Phase I studies performed at Virginia Commonwealth University (VCU) show mean ascorbate levels of 17.5 μM (normal human ascorbate levels 60 to 70 μM). Entry ascorbate levels will be drawn.

The target plasma range for modifying pro-inflammatory biomarkers and for attenuating vascular injury was obtained from the phase I safety trial and is greater than 500 μm as measured 24 hours after initiation of Vitamin C infusion. The day 2 – 7 plasma ascorbate levels are expected to be between 500 to 1000 μM based on pharmacokinetic studies generated during the phase I trial (VCU trial was entitled: Vitamin C Infusion in Human Sepsis).

Blood drawn for ascorbate levels and biomarkers will occur at hour 0 (prior to the first infusion), hour 48 (+ or – 3 hours as long as it is drawn prior to Infusion 9), hour 96 (+ or – 3 hours as long as it is drawn at least 3 hours post Infusion 16), and hour 168 (+ or – 6 hours). If patient is moved out of the ICU to another hospital unit, DO continue to collect blood for biomarkers and ascorbate levels.

If arterial line is removed prior to hour 168, do not collect arterial blood for PO2 analysis (SOFA Score component).

Completion of study drug administration: Study drug administration will be stopped when one of the following conditions is met, whichever comes first:

1. Final drug dose at 96 hours
2. Discharge from study hospital
3. Loss of indwelling venous or arterial catheter with no intent to replace the line, making it impossible to monitor glucose levels via central laboratory without multiple peripheral sticks.
4. Discharge from the ICU
5. Withdrawal from study
6. Death

Part II: Study Description Vitamin C Infusion for TReatment In Sepsis Associated Acute Lung Injury CITRIS-ALI

1. Background

1.1. Introduction

Pneumonia and extrapulmonary sepsis account for 50-65% of all ALI cases and mortality is high. Rising rates of hospitalization and death due to sepsis continue to be a worsening global health care problem.[4] A large fraction of patients with severe sepsis develop acute lung injury (ALI) or its severe form, the acute respiratory distress syndrome (ARDS).[5] The pathogenesis of ALI is characterized by activation of tissue inflammation, oxidant mediated tissue injury and increased vascular leak. At a molecular level, sepsis is associated with activation of pro-inflammatory mediators driven by transcription factor nuclear factor kappa B □NF□B). The pathogenesis of ALI is characterized by activation of tissue inflammation, oxidant mediated tissue injury and increased vascular leak. These mediators are important for host defense against invading bacteria, but their uncontrolled and excessive production ultimately contributes to the pathogenesis of ALI.

The lung is an important target of inflammatory mediators in severe sepsis[6] and increased pulmonary NF□B drives inflammatory mediators in severe sepsis.[7] Reactive oxygen species (ROS) produced by lung cells oxidize vital lung proteins and activate redox-sensitive pathological signaling pathways. An extensive body of evidence shows a crosstalk between the cellular signaling pathways and the cellular redox state through multiple mechanisms. However, exuberant ROS synthesis may also damage cells and host tissues, and, thus, contribute to the pathogenesis of ALI. Although the potential role of antioxidant enzymes and scavengers of ROS in reducing the severity of ALI has been recognized, no single agent or treatment strategy has shown sufficient promise for use in routine clinical practice.

Ascorbic acid is an essential vitamin for humans, primates, guinea pigs, and a few other animals and insects that lack the enzyme L-gulono-□-lactone oxidase. Ascorbic acid is an essential vitamin for humans, primates, guinea pigs, and a few others.[8] Ascorbic acid is transported into specialized cells as reduced ascorbic acid (AscA) by sodium dependent ascorbic acid transporters (SVCT-1 and SVCT-2) or in most cells in its oxidized form as dehydroascorbic acid (DHA) via facilitative glucose transporters.[9,10] When DHA is transported via the glucose transporters, it is rapidly reduced and trapped inside the cell, where it accumulates as reduced ascorbic acid. Although ascorbic acid circulates in normal human plasma at approximately 60 - 70 μM, it accumulates in millimolar concentrations in host defense cells.[11] Together with glutathione, AscA constitutes a primary line of defense against ROS and participates in the recycling of other antioxidants such as vitamin E. A growing body of evidence supports the notion that vitamin C is "negatively" involved in the pathogenesis of sepsis.[12] Subnormal ascorbate concentrations are common features of patients with sepsis. Furthermore, plasma ascorbate levels correlate inversely with multiple organ failure[13] and directly with survival.[14] Despite all the evidence, ascorbate is not used in a clinical setting.

At physiological pH, AscA dissociates to form dehydroascorbic ascorbate.[15] Ascorbate functions as an antioxidant by inhibiting cell death induced by hydrogen peroxide[16] and DNA damage induced by oxidative stress.[17] Ascorbate also functions as a cofactor for various enzymatic hydroxylation reactions and is involved in the biosynthesis of collagen, carnitine and norepinephrine.[18,19,20] As noted, circulating levels of ascorbate are low in patients with sepsis and plasma ascorbate correlates directly with survival and inversely with multiple organ failure. Similar results have been observed in animal models of sepsis. Ascorbate administration improves capillary blood flow, liver function and arteriolar responsiveness in experimental models of sepsis.[21,22,23] In mice injected with pathogenic bacteria, prior ascorbate depletion results in decreased survival.[24] Recently ascorbate was shown to regulate the stability of a master transcription factor HIF-1□.[25] As noted, circulating levels of ascorbate are low in patients with sepsis and plasma ascorbate may protect microvascular function by two distinct mechanisms: a) by inhibiting NADPH oxidase activation and b) by increasing endothelial nitric oxide synthase (eNOS) activity, and subsequently suppressing expression of NADPH oxidase, inducible nitric oxide synthase and tissue factor.[26] However, little is known about the effects of ascorbate administration in the setting of sepsis-mediated ALI. Gram-negative sepsis is a leading cause of ALI/ARDS and multiple organ failure.[27] Intra-peritoneal injection of a single bolus of bacterial LPS precipitates a systemic inflammatory response that resembles in many ways the observed clinical profile of sepsis including ALI and ARDS.[28] It is well known that LPS activates inflammatory cells such as polymorphonuclear leukocytes, monocytes, macrophages and lymphocytes. Besides immune cells, microvascular endothelial cells in multiple organs also become activated in sepsis and may contribute to amplification of the inflammatory response. Moreover, it is generally agreed that it is not the bacterial infection itself, but rather the inflammatory response to infection that is the predominant determinant of outcome in sepsis.[29,30] In support of this hypothesis, it has been shown that septic stimuli initiate activation of transcription factors that transactivate multiple genes such as pro-inflammatory cytokines, adhesion molecules and chemokines by endothelial cells.[31,32] Not surprisingly, efforts to block single components of the sepsis-associated inflammatory pathways have had little impact on patient survival and little progress has been made in improving outcomes.[33,34]

Summary: Sepsis is a common clinical problem that frequently leads to widespread acute vascular injury that is clinically manifested by multiple organ failure. One of the most frequent organs injured following onset of sepsis is the lung.[35] At present, no truly specific therapy is available for acute lung injury (or other organ injury) that occurs in association with sepsis. The preliminary data presented below suggests that ascorbic acid may present a means by which sepsis-associated vascular injury can be interrupted or reversed. AscA has been intravenously infused in humans in high dosages previously. Nathens et al infused 1.5 grams of AscA every 8 hours into surgical trauma ICU patients daily for 28 days with no adverse events.[36] Muhlhofer et al infused high dose ascorbic acid (7500 mg) intravenously into human volunteers daily for 7 days and showed no adverse events.[37] Finally, Tanaka et al infused high-dose ascorbic acid (66 mg/kg/hour, average 110 grams for 70 kg human) for 24 hours into patients with over 50% total body surface area burns.[38] No abnormalities in hematologic, hepatic, or renal function was associated with ascorbate infusion at day 7 following ascorbate administration. The scope of the study being presented in this phase II proof of concept

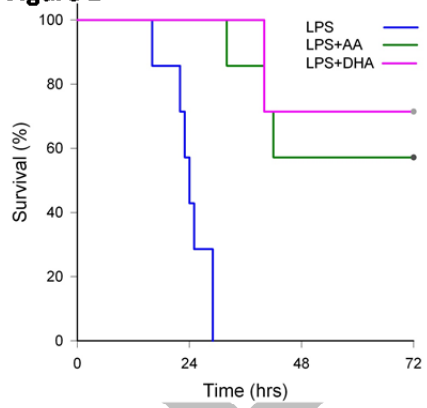
trial involves the use of intravenously administered AscA as a future potential therapy for sepsis associated acute lung injury. Previous basic scientific research currently suggests that AscA can attenuate sepsis-associated vascular injury. Further, prior data obtained from our phase I human safety studies suggests that high doses of AscA can be administered intravenously with little or no adverse events. Given these realities and the results of our phase I trials in human sepsis, we propose that intravenous AscA may present a unique therapy to improve the outcomes in human sepsis associated acute lung injury.

1.2. Preliminary Progress – Vitamin C Intervention in Experimental Septic Lung Injury

We sought to examine the biological effects of ascorbic acid (AscA) infusion on systemic inflammatory responses and acute organ injury associated with bacterial sepsis. To accomplish this, we created a durable model of septic shock and acute lung injury in wild type C57BL6 mice. Beyond the creation of an animal model system of acute lung injury, a primary goal of these studies was to determine the extent to which ascorbic acid could be employed as an ***interventional therapy*** for bacterial sepsis. Multiple prior animal studies published over the years have examined pharmacological agents (e.g., methyl prednisolone, ibuprofen, simvastatin) and biological agents (e.g., monoclonal antibody to tumor necrosis factor alpha, interleukin-1 receptor antagonist) as potential sepsis therapies. Many agents have shown efficacy in sepsis *when the agent was administered prior to induction of sepsis*. In a "real world" setting, however, any intervention for sepsis will follow the development of symptoms and altered physiology.

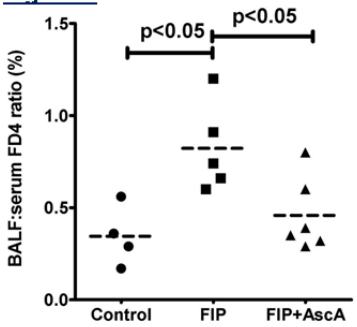
In preliminary studies described here, an interventional approach (agent administered after onset of sepsis) was employed to test the impact of ascorbic acid infusion on the course of sepsis associated acute lung injury. Sepsis was induced in mice by intraperitoneal (IP) administration of E coli lipopolysaccharide (LPS, 0111:B4) at a concentration of 10 mcg/gram of body weight. Animals had average body weights of 30 grams.

Figure 1



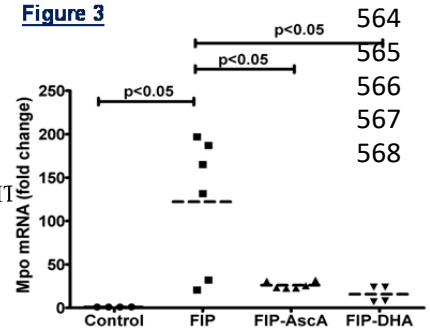
Our first goal was to examine whether AscA infusion altered the course of murine sepsis. In these studies, mice received IP LPS at the stated dose. Thirty minutes following LPS infusion, animals received intraperitoneal either the reduced form of vitamin C (AscA) or the oxidized form of vitamin C dehydroascorbate (DHA) at doses of ***200 mg/kg of body weight***. Both the reduced form and the oxidized form were employed in separate experiments. Following LPS infusion, animals were then given free access to food and water. Mortality was observed over the ensuing 60 hours. ***Figure 1*** shows Kaplan-Meier survival curves of septic mice treated with AscA and DHA. These studies show that mortality induced by E coli sepsis in wild type mice was significantly improved by both ascorbic acid forms during the 60 hour observation period.

Figure 2



Sepsis is frequently accompanied by acute lung injury (ALI). Sepsis associated ALI is characterized by acute pulmonary edema and respiratory failure. Pulmonary edema results from loss of pulmonary microvascular endothelial integrity that leads to loss of endothelial "barrier function." There is subsequent flooding of the dry airspaces of lung with plasma and cellular constituents. ALI is also characterized by intense sequestration of activated polymorphonuclear neutrophils (PMN). We assessed the loss of pulmonary microvascular barrier function using

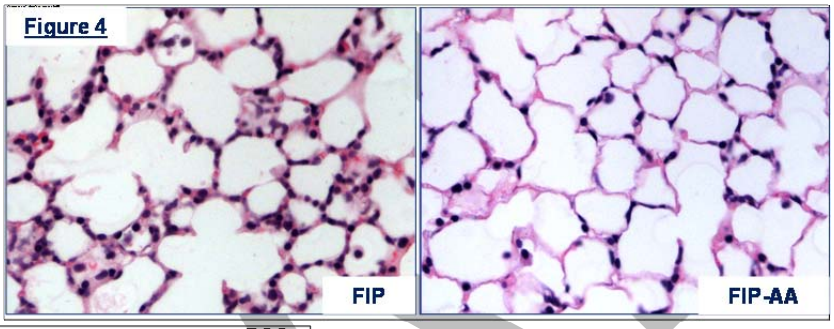
Figure 3

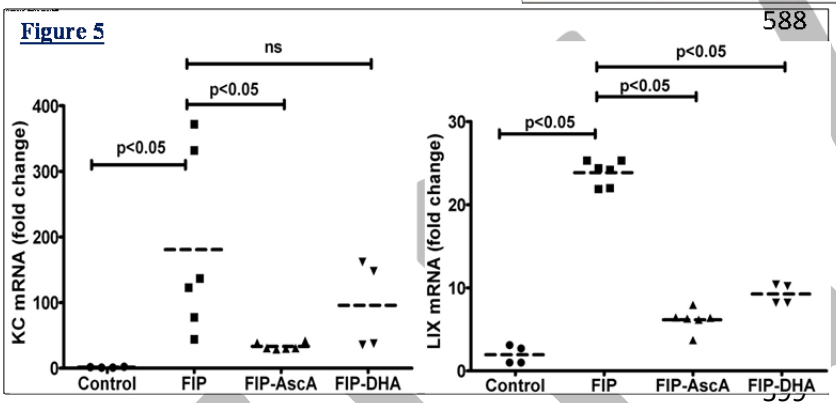


bronchoalveolar lavage fluid (BALF) protein analysis 16 hours following LPS infusion. ***Figure 2*** shows that LPS treated mice exhibit significant increases in BALF protein, indicating a loss of barrier function. AscA treatment significantly attenuated microvascular injury as assessed by BALF protein.

In a second model of sepsis (fecal induced peritonitis, FIP), lungs were removed and total RNA isolated. Quantitative real time PCR (QPCR) was performed for myeloperoxidase mRNA (surrogate for assessing the extent of PMN sequestration). ***Figure 3*** shows that untreated septic murine lung is characterized by significant PMN sequestration. Both AscA and dehydroascorbic acid (DHA) significantly attenuated PMN sequestration.

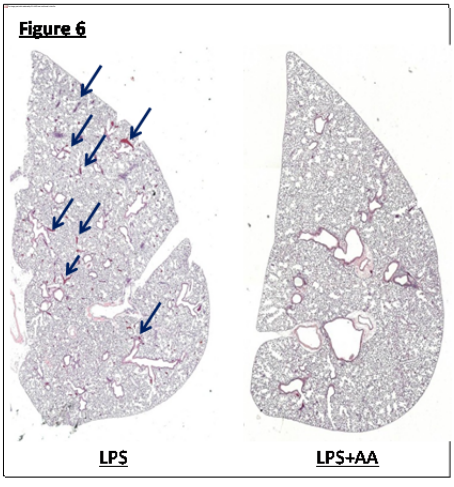
Figure 4 shows H&E stains of sections of lungs removed at 16 hours following onset of **feces induced peritonitis**. As seen in this figure, intense cellular sequestration and septal edema is present in the lung of unprotected FIP-treated mice. In contradistinction, AscA treatment significantly attenuated the histological findings of murine sepsis.

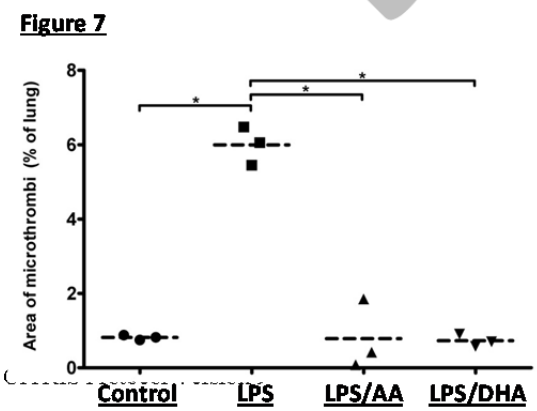




Sepsis is characterized by the development of a severe proinflammatory state with the rapid onset of expression of multiple NF□B driven genes. ***Figure 5*** shows that septic murine lung is characterized by the presence of significant expression of two nuclear NF□B activated genes examined via quantitative real time PCR (KC [murine IL-8 homologue], LIX). AscA and DHA administered following onset of sepsis significantly reduced the expression of these genes.

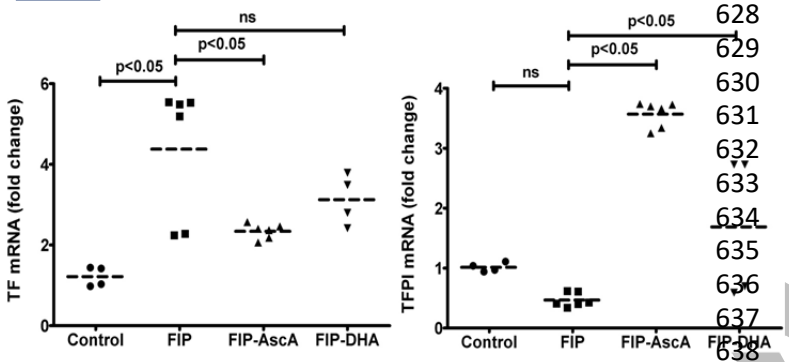
Bacterial sepsis is virtually always accompanied by disordered coagulation and is frequently associated with disseminated intravascular coagulation (DIC). A large body of significant scientific literature has documented the disruption of microvascular function/integrity induced by DIC.[39] Uncontrolled DIC uniformly induces activation of multiple proinflammatory coagulation-associated proteases that activate both intrinsic and extrinsic coagulation pathways. The resulting "cascade effect" produces widespread microvascular thrombus formation and subsequent multiple organ injury and failure. DIC frequently produces acute lung injury.[40] We examined the extent of microvascular thrombosis in the lungs of our LPS-treated wild type mice. Sixteen hours after LPS infusion lungs were fixed, paraffin embedded, and H&E stained sections examined for the presence of microthrombi. ***Figure 6***, shows that untreated septic lungs exhibit extensive microvascular thrombosis





(arrows). However, in AscA-treated septic lungs (***Figure 6***), virtually no microthrombi were observed. A Zeiss light microscope outfitted with a Axiovision counting software program was used to label and quantify microthrombi between multiple lung sections. ***Figure 7*** shows that LPS (without treatment) produced highly significant numbers of micro thromboses. AscA intervention in septic mice abolished virtually all microthrombi. Similar findings were obtained in DHA treated lungs (data not shown).

Figure 8



Following the onset of sepsis, multiple studies now show that sepsis-associated activation of ***Tissue Factor*** (factor III) is the sentinel event that induces the coagulation factor cascade leading to DIC. In further preliminary studies, we examined the expression of tissue factor in the lungs of septic mice using QPCR. ***Figure 8*** shows that both AscA and DHA dramatically attenuated the expression of tissue factor in septic lungs while inducing the inhibitor profibrinolytic peptide tissue factor pathway inhibitor (TFPI).

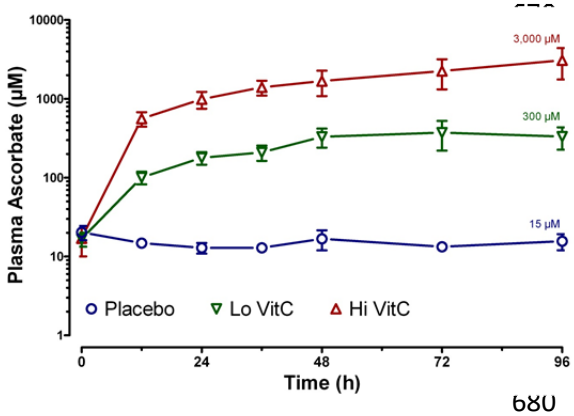
Summary Of Preliminary Animal Studies: During the animal modeling studies, *no untoward effects of AscA or DHA on animal subjects was observed*. The preliminary data shows convincingly that ascorbic acid is capable of significantly altering the course of biological events which arise following the onset of bacterial sepsis that lead to lung injury. Our results show significant impacts on sepsis induced mortality with both the reduced and oxidized forms of AscA.

1.3 Phase I Human Trial: Vitamin C Infusion In Human Sepsis: Preliminary Results From A Safety Trial

Our animal modeling studies strongly suggested that vitamin C augmentation reverses detrimental septic biology that leads to lung injury. On the strength of these studies, we proceeded with a human trial. In 2010, following IRB approval, a phase I, randomized, double blind, placebo-controlled trial testing the safety of parenteral vitamin C in patients with severe sepsis was initiated at the VCU Medical Center. All patients enrolled, regardless of study arm, received full ICU standard of care. Patients were randomized to placebo (5% dextrose/water, D5W), low dose vitamin C (50 mg/kg/24hr), or high dose vitamin C (200 mg/kg/24hr). The calculated 24 hour vitamin C dose was divided into four equal doses and administered intravenously (in 50 ml D5W) over 30 minutes every 6 hours for 96 hours. Vital signs were monitored every 5 minutes during infusion and for 45 minutes afterwards by bedside ICU Nursing and the investigative team. ***Hypotension***, ***tachycardia***, and ***nausea/vomiting and hypernatremia*** were the primary safety outcomes assessed. A multi-departmental data safety monitoring board oversaw patient enrollment into the trial. Serum/plasma specimens were obtained every twelve hours for 2 days, then once daily for two days. ***Enrollment:*** Over a 1 year period, twenty-four patients were randomized to the three groups (*Placebo*, 4M, 4F, age 54-68 yrs.), (*Lo-VitC*, 5M, 3F, age 30-70 yrs.), (*Hi-VitC*, 4M, 4F, age 44-92 yrs.). ***APACHE II score at Enrollment:*** Mean APACHE II scores between groups were: *Placebo* - 20.4 (range: 15-29), *Lo-VitC*: 20.2 (range: 12-33), and *Hi-VitC*: 24 (range: 17-33) respectively. The groups were statistically identical.

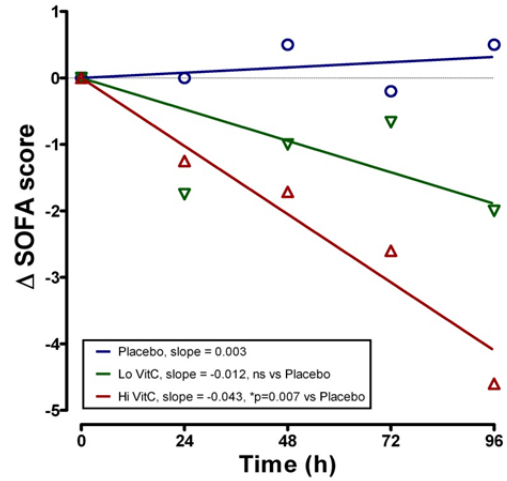
Phase I Primary Outcomes: Safety of Vitamin C Infusion: Safety of vitamin C infusion was the primary focus of this trial. During the 96 hour infusion period, no patients were withdrawn due to identified negative outcomes (hypotension, tachycardia, nausea/vomiting, or hypernatremia). Infusions were halted in one patient (Hi-VitC) following infusion #14 (80hrs) for a ventricular arrhythmia later determined by Cardiology to be artifact. This patient is included in the analysis. One patient (Hi-VitC) was transferred to another facility at 48 hours at the insistence of family and was lost to follow up.

Figure 9



Plasma Ascorbate Levels in Human Sepsis: The impact of Vitamin C Infusion: Vitamin C levels were quantified by HPLC in all patients at enrollment then at defined intervals to 96 hours. Ascorbate levels in all septic patients at enrollment were subnormal (hyposcorbic) at 17.9 ± 2.4 μM (normal 50 – 70 μM) and were not significantly different at baseline. **Figure 9** shows the change in plasma ascorbate levels through time across patient groups. Ascorbate levels rose rapidly in the two treatment groups and were significantly higher than placebo within twelve hours (Lo-VitC vs. placebo P<0.005, Hi-VitC vs. placebo p<0.0005) remaining consistently elevated for 96 hours. Ascorbate levels in the Hi-VitC group were significantly higher than the Lo-VitC group from the 12 hour point forward. These data show that an intermittent ascorbate infusion protocol (every 6 hour) produces sustained steady state levels.

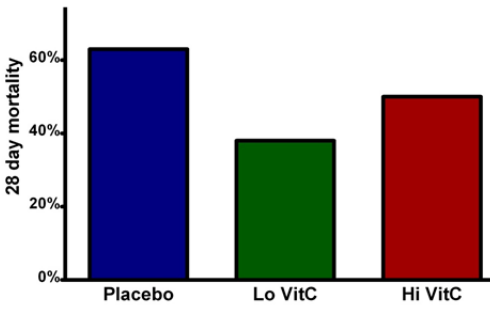
Figure 10



Sequential Organ Failure Assessment (SOFA) Scores SOFA scores obtained are robust indicators of mortality during critical illness.[41] Increases in SOFA scores during the first 48 hours of ICU care predicts a mortality rate of at least 50%. Initial SOFA scores at enrollment were: placebo – 13.3 ± 2.9, Lo-VitC – 10.1 ± 2.0, and Hi-VitC 10.8 ± 4.4 and were not significantly different across groups. ***Figure 10*** shows that patients treated with high dose of vitamin C exhibited significantly lower SOFA scores when compared to placebo over the 96 hour period. SOFA scores among treated patients did not exhibit any subsequent rise whereas patients in the placebo group exhibited a gradual rise in scores. Though the cohort size is limited, these early data suggest that vitamin C infusion attenuates systemic organ injury associated with sepsis.

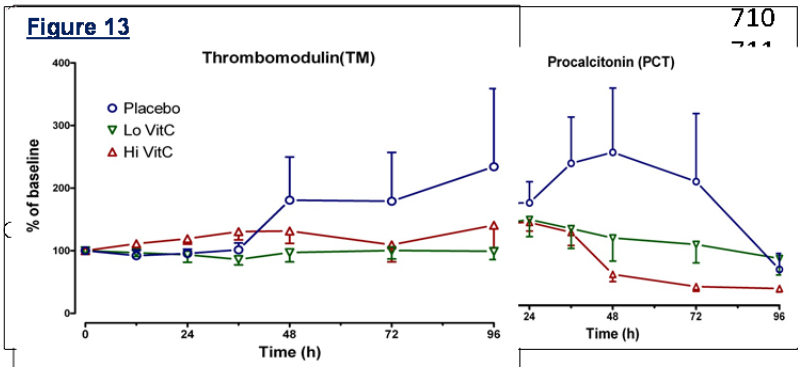
Phase I Trial Secondary Outcomes: Following enrollment 1) Days on vasopressor (DOVP), 2) ventilator free days (VFD), and 3) ICU days (ICUD), were monitored as secondary outcomes. We observed trends for fewer DOVP and ICUD and more VFD in the Lo-VitC patients, but the numbers were small and statistically insignificant. Though this study was not powered to assess mortality, we present the results of 28 day all-cause mortality as a prospectively identified secondary outcome (**Fig 11**). In the placebo group we found a 63% mortality (5 of 8 patients died). In the Lo-VitC, 3 of 8 patients died for 38% mortality, and 4 of 7 patients died in the Hi-VitC for a mortality of 57%. The data showed in figure 11 further attests to the safety of the vitamin C dosing regimens in that there was no additional added mortality among the treatment group patients when compared to placebo.

Figure 11



Vitamin C Infusion Attenuates Biomarkers of Inflammation and Endothelial Injury In Patients with Severe Sepsis Sera obtained from enrolled subjects was analyzed for three biomarkers: C-reactive protein (CRP), procalcitonin (PCT), and thrombomodulin (TM). CRP and PCT were quantified as surrogates for inflammation while TM was employed as a surrogate for endothelial injury. At enrollment, biomarker levels across the three groups were not

Figure 13



significantly different. CRP and PCT levels in all patient groups started high and trended down over time (**Fig 12**). Of importance, patients randomized to receive low or high dose vitamin C exhibited more rapid reductions in PCT and CRP levels than patients randomized to placebo, achieving significantly lower levels when compared to their own baseline by 48 hours ($p<0.05$). Thrombomodulin levels in patients randomized to placebo, though not different at baseline, began increasing, becoming significantly elevated beyond 36 hours remaining significantly elevated when compared to vitamin C treated patients (**Fig 13**). Vitamin C treated patients did not exhibit the increases in TM levels observed in placebo-infused patients. Our preliminary results suggest for the first time that vitamin C infusion produces early reductions in proinflammatory mediators in patients with severe sepsis. The results further suggest that vitamin C infusion

Summary of Preliminary Phase I Safety Studies in Human Sepsis: During phase I human study/trial no untoward/adverse effects of intravenous vitamin C infusion were observed in any patient during the 96 hour treatment protocol. The preliminary data gathered suggests that vitamin C is capable of significantly altering the course of organ failure, which arises in humans following the onset of bacterial sepsis. Biomarker data also suggest that vitamin C infusion attenuates proinflammatory peptide expression, a process that contributes to sepsis mediated acute lung injury. Further, ascorbic acid infusion prevented subsequent increases in plasma thrombomodulin (an indicator of vascular injury).

1.4. Potential Mechanisms of Action of Ascorbic Acid in Sepsis

- Our experimental data in two murine models of sepsis induced lung injury suggest that vitamin C when infused acts in a pleotropic manner, attenuating NF□B inducible genes (chemokines, tissue factor) while boosting expression of genes leading to active fibrinolysis (i.e., tissue factor pathway inhibitor).
- Humans lack L-gulono-γ-lactone oxidase, the final enzyme in vitamin C biosynthesis.[42]
- Sodium-dependent vitamin C transporters move vitamin C into cells in reduced form or via facilitative glucose transporters in oxidized form as dehydroascorbic acid (DHA).[43] DHA is rapidly reduced and trapped intracellular as reduced vitamin C or L-ascorbic acid.
- Though vitamin C circulates in normal human plasma at 60-70μM, it accumulates normally in ***millimolar concentrations in host defense cells (i.e., neutrophils, platelets, macrophages) and endothelium***.[44] Together with glutathione, vitamin C constitutes a primary line of defense against ROS and promotes recycling of other antioxidants (e.g., vitamin E).
- Subnormal plasma vitamin C concentrations in septic patients correlate inversely with multiple organ failure and directly with survival. Vitamin C depletion in sepsis results from: 1) ascorbate consumption by reduction of plasma free iron, 2) ascorbate consumption by the scavenging of aqueous free radicals, and 3) by destruction of DHA.[45] Sepsis associated vitamin C destruction permits uncontrolled oxidant activity.
- Clinical protocols currently in use for hospitalized septic patients are inadequate to normalize plasma vitamin C levels.[46]
- Ascorbate infusion into septic animals: 1) improves survival, corrects hypotension,[47] improves capillary blood flow, [48] protects endothelial barrier function,[49] attenuates peroxynitrite formation,[50] attenuates ALI, and disrupts lung capillary microvascular thrombosis (see preliminary murine data above).

1.5. Ascorbic Acid Dose Selection

Dosing and bio-distribution data in humans show that pharmacological concentrations of vitamin C can only be attained following intravenous administration.[51] Dosage selection for this trial was determined both from animal modeling, examining the biological effectiveness in a lung injury model system and from the recently conducted randomized double blind phase I human sepsis safety trial. The 200 mg/kg/24 hour IV dosing protocol was determined from quantification of plasma ascorbate levels and from assessing the impact on SOFA scores. Further, the dosage was selected following observation of the 200 mg/kg/24 hour regimen on biomarker levels.

1.6. Study Rationale

The purpose of this study is to assess the efficacy of intravenously infused ascorbic acid therapy for patients with sepsis associated ALI. By restricting the population to those we believe to have both infection and evidence for organ dysfunction (severe sepsis), this study targets a disease process and population that has been best studied in animal models and by a small RCT. By focusing on sepsis associated ALI, we have selected a group that has a higher disease burden than sepsis alone and thus likely to have both increased mortality and an increased opportunity for benefit, including a reduction in the requirement for mechanical ventilation. Given that the mortality and ventilator days are significant in patients with sepsis associated ALI, we believe there is real opportunity for improved clinical outcomes if the right interventional agent can be identified. In choosing the SOFA scores, and biomarkers of inflammation, vascular injury, and coagulation, as the primary outcomes, we will be able to detect changes in clinical outcomes that are important for proving proof of concept.

2. Objectives

2.1. Primary Objectives

To assess the efficacy of a 96-hour high dose intravenous vitamin C infusion protocol (200 mg/kg per 24 hours) in patients with established ALI/ARDS that results from severe sepsis. Patients will be randomized to receive either: 1) ***Placebo*** (50 ml of 5% dextrose in water) or ***Vitamin C*** (sterile L-ascorbic acid for injection at 200 mg/kg per 24 hours with entire calculated 24 hour dose diluted in 200 ml of 5% dextrose in water). One fourth of the 24 hour calculated dosage will be administered in 30 minute intravenous infusions will occur every 6 hours.

Clinical, physiological, and biomarker data will be collected at various time points while on study (See Appendix G). All data collected below will occur at the following timepoints: hour 0 (collected within the 24 hours prior to randomization, or post randomization, but pre-infusion), hour 48 (a timepoint prior to infusion 9), 96 (a timepoint close to hour 96 and after infusion 16), hour 168 (a timepoint close to hour 168)

:

- VS (Body weight, blood pressure, heart rate, Temperature, mean arterial pressure, oxygen saturation, central venous pressure, glasgow coma score)
- Vasopressor use (amount and type) (mcg/kg/min)
- Ventilator data – Is the patient on or off vent?
  - ✓ <u>If on mechanical ventilation</u>: Mean Airway Pressure, tidal volume, Peak Inspiratory Pressure, FiO2, Respiratory Rate, Plateau Pressure, Positive End Expiratory Pressure (data recorded from chart at the 8am time point or closest time point to 8am available)
- Arterial Blood Gases: pH, PaO_2, $PaCO_2$, SpO_2 - for as long as subject has arterial line
- Laboratory (Sodium, Potassium, Chloride, Metabolic Glucose, Hemoglobin, Hematocrit, Platelets, White Blood Cell Count, Creatinine, Blood Urea Nitrogen, Bilirubin (data recorded from chart at the 8am time point or closest time point to 8am available).
- Biomarker blood sample
- **S**equential **O**rgan **F**ailure **A**ssessment **S**core (SOFA): (if intubated use PaO2/FiO2 ratio for calculation. If not intubated see Appendix H for caluculating FiO2 and PaO2/FiO2 from SpO2. Calculate using the worst value in the 24 hours preceding the Score time point for each component. See Appendix G for Modified SOFA Score Calculator.

2.2. Hypothesis

Hypothesis 1A: Vitamin C infusion will significantly attenuate sepsis associated systemic organ failure as measured by Sequential Organ Failure Assessment (SOFA) score,

Hypothesis 1B: Vitamin C infusion will attenuate sepsis associated lung injury as assessed by the oxygenation index and the VE40

Hypothesis 1C: Vitamin C infusion will attenuate biomarkers of inflammation (C-Reactive Protein, Procalcitonin), vascular injury (Thrombomodulin, Angiopoietin-2), alveolar epithelial injury (Receptor for Advanced Glycation End Products), while inducing the onset of a fibrinolytic state (Tissue Factor Pathway Inhibitor).

3. End-Points

Analysis of the primary, secondary and other endpoints will be conducted on an intention-to- treat (as randomized) basis.

3.1. Primary Endpoints

Primary Endpoint #1: Change in SOFA score at 96 hours as compared to baseline when compared to placebo.

Primary Endpoint #2: C-Reactive Protein and Thrombomodulin at study hours 0, 48, 96, 168 when compared to placebo.

Explanation For The Choice of Primary Endpoints: The phase I trial was a safety trial and numbers of patients studied were small. The SOFA score was chosen as the **"physiological primary endpoint"** due to the prompt and significant reductions in the SOFA score observed in the high dose vitamin C group. The SOFA score, though not a primary lung function score, contains the PaO_2/FiO_2 ratio in its calculation. We have chosen C-Reactive Protein and Thrombomodulin as broad indicators of inflammation and vascular injury to serve as the primary endpoint biomarkers.

3.2. Secondary End Points

Secondary Endpoints:

- Oxygenation Index (FiO_2 x Mean Airway Pressure/PaO_2) at study hour 0, 48, 96, 168 if still intubated in ascorbate infused patient compared to placebo.
- VE-40 (Vent RR x TV/Weight) x ($PaCO_2$/40) at study hour 0, 48, 96, 168 if still intubated, in ascorbate infused patient compared to placebo
- SOFA scores at hours 48, 96, 168
- SOFA Score Components at hours 48,96, 168
    - PaO2/FiO2
    - SpO2/FiO2
    - Platelets
    - Total Bilirubin
    - Vasopressor status
    - GCS
    - Creatinine or Urine Output
- Angiopoietin-2, Procalcitonin, Receptor for Advanced Glycation End Products, Tissue Factor Pathway Inhibitor at study hour 0, 48, 96, 168
- Ascorbate level at hour 0, 48, 96, 168
- Ventilator Free Days to day 28

- ICU-free days at day 28
- All cause mortality to day 28
- Hospital-free days at day 60

VE40 is a bedside pulmonary dead-space calculation and is defined as the minute ventilation needed to bring $PaCO_2$ to 40 mm Hg.[52] Ventilator Free Days or VFDs to day 28 are defined as the number of days from the time of initiating unassisted breathing to day 28 after randomization, assuming survival for at least two consecutive calendar days after initiating unassisted breathing and continued unassisted breathing to day 28. If a patient returns to assisted breathing and subsequently achieves unassisted breathing to day 28, VFDs will be counted from the end of the last period of assisted breathing to day 28. A period of assisted breathing lasting less than 24 hours and for the purpose of a surgical procedure will not count against the VFD calculation. If a patient was receiving assisted breathing at day 27 or dies prior to day 28, VFDs will be zero. Patients transferred to another hospital or other health care facility will be followed to day 28 to assess this endpoint. ICU- and Hospital-free days to day 28 and day 60 are defined as the number of days alive between day 1 and day 28 and day 1 and day 60 which were spent outside the ICU or outside of the hospital respectively.

3.3. Focused Safety Analysis:

The current trial will be enrolling patients with sepsis associated acute lung injury. We therefore expect that many of these patients will have some degree of organ dysfunction.

3.3.1 Renal Monitoring Plan

Patients with sepsis are at high risk of metabolic acidosis (including lactic acidosis). To prevent the possibility of metabolic acidosis due to drug administration, the study drug is formulated to a neutral pH of 7.4. Therefore, we do not anticipate the need for additional monitoring of acid/base balance beyond standard-of-care provided at each institution. Any observed abnormalities will be evaluated according to standard-of-care practice and documented in the research record.

3.3.2 Glucose Monitoring Plan

Guidance for blood glucose monitoring in patients enrolled in the CITRIS-ALI Trial:

Ascorbic acid is known to artefactually **raise** POC blood glucose readings by all POC devices except the StatStrip glucometer. However, it does not raise blood glucose readings from a basic metabolic panel or glucose results using the gas lab. Thus, extreme care must be taken to assure an accurate blood glucose level from a metabolic laboratory (BMP) or arterial blood gas panel before initiating any insulin therapy, including sliding scale or scheduled insulin.

All study sites not using the StatStrip POC glucometer should follow these guidelines:

Guidance for blood glucose monitoring in patients enrolled this study:

- Critical care Nursing and Physician leadership at all study sites must be informed of vitamin C's effect on point of care (glucometer) blood glucose and arterial blood gas glucose point of care values.
- In-service training will be documented in the Study Training Log
- Bold signage will be displayed on all study instructions, data collection forms, and at the patient's head of bed, stating:
  - STOP! Do not use Accuchek or other Point of Care devices to measure glucose on this patient
  - Use only metabolic or gas lab glucose screening methods
  - This patient is enrolled in a study with Vitamin C, which artefactually increases POC glucose testing

- Do Not Initiate or Utilize Sliding Scale, Scheduled Insulin, or Continuous Insulin Infusion Without Laboratory Confirmation of Blood Glucose

- Those receiving insulin infusion or sliding scale insulin will have metabolic glucose screening on the schedule determined by the primary physician and paid for by the study
- Blood glucose monitoring for insulin administration guidance should *only* be by a metabolic or blood gas laboratory measured blood glucose results, whether or not the study patient is receiving insulin
- Study personnel will follow each study patient closely to monitor insulin use to ensure that point of care glucose screening is suspended for the research subject.
- If subject loses central venous access (PICC line and arterial line acceptable), Vitamin C infusions are to stop but subject not withdrawn. Data collected through end of study.
- Point of care glucose testing may resume 36 hours after the last infusion of study drug.

4. Study Population and Enrollment

4.1. Number/Source/Screening

The trial will accrue a maximum of 170 patients over a 2-3 year period. Patients with sepsis associated ALI will be recruited from intensive care units at Virginia Commonwealth University Health System, Medical College of Wisconsin and sub-site, Aurora St. Luke's Medical Center, The Cleveland Clinic Health System and its sub-site, Fairview Hospital. Study personnel will review patients within intensive care units daily to identify potential candidates for enrollment. Permission to approach patients and/or their families will be requested from the attending physicians in charge of patient care in the ICU. All patients meeting the inclusion/exclusion criteria will be approached with a consent and will be entered into a screening log. If the patient is not enrolled, the screening log will include information explaining why enrollment did not occur (exclusion criteria, attending physician denial, patient refusal, etc.).

Patients will be documented in the Study Screening Log when all Inclusion Criteria are met. A Screen Failure is defined as a patient meeting all Inclusion Criteria but not meeting Exclusion Criteria.

4.2. Inclusion Criteria

4.2.1 CITRIS-ALI Inclusion Criteria:

CITRIS-ALI Inclusion Criteria:

Patients must have suspected or proven infection, and meet 2 out of 4 of the criteria for Systemic Inflammatory Response (SIRS) due to infection, and be accompanied by at least 1 criterion for sepsis associated organ dysfunction, *and* meet all 5 criteria for Acute Respiratory Distress Syndrome (ARDS).

1. *Suspected or proven infection:* (e.g., thorax, urinary tract, abdomen, skin, sinuses, central venous catheters, and central nervous system, see Appendix A).
2. *The presence of a systemic inflammatory response*: Defined as: *fever:* >38ºC (any route) or *hypothermia:* <36ºC (core temp only), *tachycardia:* heart rate > 90 beats/min or receiving medications that slow heart rate or paced rhythm, *leukocytosis*: >12,000 WBC/µL or *leukopenia*: <4,000 WBC/µL or >10% band forms. Respiratory rate > 20 breaths per minute or PaCO2 < 32 or invasive mechanical ventilation.
3. *The presence of sepsis associated organ dysfunction:* (any of the following thought to be due to infection)
    a. Sepsis associated hypotension (systolic blood pressure (SBP) < 90 mm Hg or an SBP decrease > 40 mm Hg unexplained by other causes or use of vasopressors for blood pressure support (epinephrine, norepinephrine, dopamine =/> 5mcg, phenylephrine, vasopressin)
    b. Arterial hypoxemia (PaO_2/FiO_2 < 300) or supplemental O2 > 6LPM.
    c. Lactate > upper limits of normal laboratory results
    d. Urine output < 0.5 ml/kg/hour for > two hours despite adequate fluid resuscitation

e. Platelet count < 100,000 per mcL
f. Coagulopathy (INR > 1.5)
g. Bilirubin > 2 mg/dL
h. Glasgow Coma Scale < 11 or a positive CAM ICU score

4. *ARDS characterized by all the following criteria*
   a. Lung injury of acute onset, within 1 week of an apparent clinical insult and with progression of respiratory symptoms
   b. Bilateral opacities on chest imaging not explained by other pulmonary pathology (e.g. pleural effusions, lung collapse, or nodules)
   c. Decreased arterial PaO2/FiO2 ratio ≤ 300 mm Hg
   e. Minimum PEEP of 5 cmH_2O

CITRIS-ALI Exclusion Criteria:

1. Known allergy to Vitamin C
2. inability to obtain consent;
3. age < 18 years;
4. Not on a ventilator
5. No indwelling venous or arterial catheter in patients requiring insulin in a manner that requires glucose being checked more than twice daily (e.g. continuous infusion, sliding scale)
6. Presence of diabetic ketoacidosis
7. more than 48 hrs since meeting ARDS criteria;
8. patient or surrogate or physician not committed to full support (not excluded if patient would receive all supportive care except for cardiac resuscitation);
9. pregnancy or breast feeding,
10. moribund patient not expected to survive 24 hours;
11. home mechanical ventilation (via tracheotomy or noninvasive) except for CPAP/BIPAP used only for sleep-disordered breathing;
12. on home O2 > 2LPM, except for with CPAP/BIPAP
13. diffuse alveolar hemorrhage (vasculitis);
14. interstitial lung disease requiring continuous home oxygen therapy;
15. Active kidney stone
16. Non English speaking;
17. Ward of the state (inmate, other)

4.3. Enrollment, Randomization, and Study Initiation Time Window

All ALI criteria (4.2.3 a-d above) must occur within the same 24-hour period. The onset of ALI is when the last criterion is met. Patients must be consented within 48 hours of ALI onset. SIRS criteria must occur within the 48 hours before and 24 hours after ALI onset. Information for determining when these time window criteria were met may come from either the study hospital or a referring hospital report. Randomization must occur within the same 48 hours of ALI onset, as is for consent. Dose 1 must be administered within 6 hours of randomization. Following randomization, the low tidal volume protocol for mechanical ventilation (Appendix C) and the fluid management strategy protocol (Appendix D) may be initiated within one and four hours respectively (if not already being utilized), if clinically indicated.

4.4. Informed Consent

Informed consent will be obtained from each patient or surrogate (family or legal representative) before enrollment in the trial. No study procedures will be conducted before obtaining informed consent.

4.5. Randomization

After informed consent is given, a randomized assignment will be made by the *Investigational Pharmacy* of the Lead Site and Coordinating Center (VCUHS) to administer either Vitamin C therapy or placebo. Each participating pharmacy will have a pre-defined randomization chart by which to determine whether to administer the study drug or placebo to each particular subject. The randomization will be stratified by institution to one of the two study arms.

Table I Demographics of CITRIS-ALI Study Sites*

	Atlanta	Cleveland	Charlottesville	Richmond	Average
Female	50.2%	53.0%	52.3%	52.3%	51.7%
Male	49.8%	47.0%	47.7%	47.7%	48.3%
Hispanic	5.2%	14.7%	5.1%	6.3%	7.8%
Not Hispanic	94.8%	85.3%	94.9%	93.7%	92.2%
American Indian	0.2%	0.4%	0.3%	0.3%	0.3%
Asian	3.1%	2.0%	6.4%	2.0%	3.4%
Native Hawaiian	0.1%	0.1%	0.1%	0.1%	0.1%
African American	54.0%	34.7%	19.4%	50.6%	39.7%
White	38.4%	51.2%	69.1%	40.0%	49.7%

*Source: United States Census Bureau (http://2010.census.gov/2010census

4.7. Minorities and Women

The demographic profiles of the Centers selected for the study show that the aggregate patient population contains representative proportions of minorities and women (Table 1). Recruitment of minorities and women will be monitored by the Study Coordinating Center. If necessary, additional recruitment efforts will be made at specific centers to ensure that the aggregate patient sample contains appropriate gender and minority subsets. Pregnant women will be excluded because of the lack of safety data for infused Vitamin C use during pregnancy.

5. Study Procedures

If a pregnancy test is not available before informed consent, blood or urine tests will be obtained after informed consent but before randomization to ensure eligibility. Patients excluded on the basis of tests obtained in this manner will not be included in the study.

5.1. Vitamin C or Placebo Administration

All study drug doses will be administered via central or peripheral line infusion. Should no central or peripheral line be available at scheduled time of infusion, a call should be placed to pharmacy to determine if study drug may be piggybacked into the line that is infusing a different drug. If administering study drug via piggyback is contraindicated then study drug infusion may be delayed by a maximum of 6 hours. If clinical drug administration schedule is such that study drug will not have an available administration time beyond this delay, a dedicated new line (peripheral or central) should be inserted. Study drug will be blinded using an identical appearing placebo.

The prepared IV bags will have the IV tubing attached and primed by the study pharmacist. Amber shrouding will be used to cover the IV bag and the IV tubing in order to maintain the blind. The product labeling will be blinded as to what the actual product is. For example, the drug name and dose will be indicated as per the following: "Ascorbic acid ____mg or placebo in 50cc D5W".

1. **First study drug dose** (L-ascorbic acid or placebo) will be considered “Dose 1” and will be administered within 6 hours of randomization or the earliest available time post any clinically indicated procedure which requires the patient to be off the unit. All doses will be administered in the ICU. Patients receiving vitamin C will receive 25% of the total daily calculated dosing (200mg/kg/24 hours) and will be infused over 30 minutes for this first dosing.
2. **Subsequent doses** which represent 25% of the day’s total dose will be infused every six hours through 96 hours (+/- 3 hours).

a. Timing of Dose 2 will be triggered by the physician order for q 6 hour administration and will therefore be listed on the bedside MAR. As such, timing of Dose 2 may be out of the +/- 3 hour window and will not trigger a protocol deviation.
b. If for any reason any other maintenance dose is not administered within window, the dose will be skipped and the next scheduled dose will be given and documented in the data collection tool.

5.2. Drug Level Specimens (venous blood)

Preliminary studies performed during the phase I trial showed that an every 6 hour infusion protocol resulted in steady state plasma levels after 18 hours. In this phase II trial, plasma levels of Vitamin C will be obtained to determine the relationship, if any, of plasma levels to either the pleiotropic effects or the toxicities of Vitamin C or to biomarker levels.

5.3. Completion of Study Drug Administration

Completion of study drug administration: Study drug administration will be stopped when one of the following conditions is met, whichever comes first:

1. Final drug dose at 96 hours or discharge from ICU, whichever comes first.
2. Discharge from study hospital
3. Loss of indwelling venous or arterial catheter with no intent to replace the line, making it impossible to monitor glucose levels via central laboratory without multiple peripheral sticks.
4. Withdrawal from study
5. Death

Note: If a patient is readmitted to the ICU after study drug has already been stopped per protocol, it does **NOT** get restarted when readmitted to the ICU.

5.4. Premature Withdrawal from Treatment

Loss of indwelling venous or arterial catheter will trigger the stopping of Vitamin C infusions but subjects will remain on study. Blood glucose monitoring will continue via the central laboratory for 36 hours after the last infusion via peripheral IV draws or peripheral sticks. Biomarker sampling via peripheral IV and/or peripheral stick is allowable as it occurs only 4 times throughout the study and likely only once (if at all) after the patient has been discharged from the unit and is without a central line.

The study drug will be discontinued if a patient develops a metabolic acidosis unexplained by other etiologies (lactic acidosis secondary to septic shock). Determination of the presence of metabolic acidosis will be made by the site investigator. Study drug will also be discontinued if primary care team or surrogate decision maker request withdrawal. Data collection will continue on these patients following withdrawal of study drug.

Requests to unblind a patient's study treatment can be made to the study (investigational) pharmacist. Unblinding study treatment should occur only in the case of an emergency when knowledge of the study treatment is essential for subject care to treat a serious adverse event and prevent further harm or death.

If possible, a decision to unblind should be discussed with the Principal Investigator or a sub-Investigator prior to unblinding the study treatment.

If a blind is broken *(either intentionally or unintentionally)*, the circumstances should be documented as to who, what, when, and why and all documentation shall be kept by the study pharmacist.

5.5. Ventilator Procedures

Ventilator management, including weaning, will follow the modified ARDS Network lower tidal volume (6 ml/kg PBW) protocol (Appendix C).[53] If not already being utilized, this low tidal volume protocol for mechanical ventilation will be initiated within one hour of randomization, if possible. Since the time a patient achieves unassisted ventilation affects the secondary endpoint of ventilator free days (VFDs), and because recent evidence-based consensus recommendations have identified a best practice for weaning, weaning strategy will also be controlled by protocol rules in accordance with these evidence-based recommendations. This will assure similar weaning methods. This newer weaning strategy is a simplified version of the weaning strategy used in prior ARDS Network study protocols (see Appendix C).

5.6. On-Study Fluid Management

Fluid management during shock will not be prescribed per study protocol. In subjects who are not in shock, a conservative fluid management approach will be administered, if possible. This conservative fluid management approach will represent a simplification of the algorithm utilized in the ARDS Network FACTT study (see Appendix D).[54] If not already being utilized, this conservative fluid management approach will be initiated, if possible, within four hours of randomization and continued until the subject has reached unassisted breathing (UAB) or study hour 168, whichever occurs first.

6. Data Collection

6.1. Background Assessments

1. Demographic:
    a. Gender
    b. Age
    c. Race/Ethnicity
2. Insurance status
    a. Privately insured
    b. Medicaid
    c. Medicare
    d. Other public
    e. uninsured

3. Pertinent Medical History and Physical Examination
    a. Etiology of Sepsis
    b. diabetic status – Hx of Diabetes ☐ Yes ☐ No
        i. Insulin received? ☐ Yes ☐ No
    c. patient place of residence: Home independently, home with help (supervision, direction, personal assistance), home with professional help (nursing/nursing service), intermediate care or rehab facility, skilled nursing facility, other (specify)
    d. Patient admitted directly from: OR, Recovery Room, ER, Floor, another special care unit, another hospital, direct admit, step-down unit
    e. Hx of alcohol use via the AUDIT-C Questionnaire:
        i. How often do you have a drink containing alcohol?
        ☐ Never
        ☐ Monthly or less
        ☐ 2-4 times per month

☐ 2-3 times per week
☐ 4 or more times per week

ii. How many standard drinks containing alcohol do have on a typical day?

☐ 1 or 2
☐ 3 or 4
☐ 5 or 6
☐ 7 to 9
☐ 10 or more

iii. How often do you have six or more drinks on one occasion?

☐ Never
☐ Less then monthly
☐ Monthly
☐ Weekly
☐ Daily or almost daily

4. Study enrollment date
5. Acute or Chronic renal failure and use of dialysis

6.2. Baseline/Hour 0 Assessments and Procedures

The following information will be recorded during the 24 hour interval preceding randomization. If more than one value is available for this 24 hour period, the value closest to the time of randomization will be recorded. If no values are available from the 24 hours prior to randomization, then values will be measured post randomization but prior to initiation of study drug.

Vital Signs: Blood Pressure (BP), Heart Rate (HR), Mean Arterial Pressure (MAP), Respiratory Rate (RR), Temperature., O_2 saturations, Central Venous Pressure (CVP), Body weight, Glascow Coma Score
Suspected or known site of sepsis
SOFA Score
V_E40 = [Minute Ventilation ÷ Weight (kg)] x [$PaCO_2$ ÷ 40] – closest one to time of randomization
Oxygenation Index = [F_iO_2 x Mean Airway Pressure] ÷ PaO_2
Ventilator Data: tidal volume, FiO_2, PEEP, inspiratory plateau pressure, Peak Inspiratory Pressure, and mean airway pressures.
Arterial Blood Gasses: PaO_2, $PaCO_2$, pH, HCO3 and SpO_2
Serum Sodium, Potassium, Metabolic Glucose, BUN, Creatinine, Billirubin Total, WBC, Hgb, Hct, Platelets, PT/INR
Vasopressors or inotropes (epinephrine, norepinephrine, phenylephrine, vasopressin, dopamine)
Insulin received? ☐ Yes ☐ No
In/Out – total – for first 7 days or for every day in ICU, whichever is shorter
In/Out – Urine - for first 7 days or for every day in ICU, whichever is shorter
Concomitant Medications: use of steroids
Blood for biomarkers will equal approximately 12ml. Blood samples will be processed and divided per the Laboratory Instructions Manual

6.3. Assessments after Enrollment

The following data will provide the basis for assessing protocol compliance and safety as well as between-

group differences in several efficacy variables. Data for each of the variables will be recorded on the days shown in the Time-Events schedule (Appendix E) or until death or discharge from the intensive care unit.

Hours:	Required:
Q 24 hours for as long as patient is in ICU or 7 days (whichever comes first	I/O Total I/O Urine Insulin Receipt
0, 48, 96, 168	VS, SOFA, ABGs, Routine Labs, Con Meds, AE/SAE Assessments, Blood draw for Biomarkers, Insulin Receipt As Available: VE40, OI, Weight, Bilirubin, Ventilator Data, known site of sepsis
28 Days	Required: Ventilator Free Days to Day 28, All-Cause Mortality to Day 28, ICU Free Days to Day 28 Use of dialysis
60 Days	Hospital Free Days at Day 60

Reference Measurements

All data collected below will occur at the following timepoints: hour 0 (collected within the 24 hours prior to randomization, or post randomization, but pre-infusion), hour 48 (a timepoint prior to infusion 9), 96 (a timepoint close to hour 96 and after infusion 16), hour 168 (a timepoint close to hour 168)

1. Ventilator Data - The following conditions will be ensured prior to measurements: no endobronchial suctioning for 10 minutes; no invasive procedures or ventilator changes for 30 minutes.
2. Arterial Blood Gases when Arterial line is in place for clinical reasons
3. Vital Signs
4. Labs
5. Vasopressor
6. Use of Steroids
7. Blood samples for biomarkers.

Blood specimens will be batch-sent to the VCU Central Repository to be stored. Specimens will be identified by a unique number. All data released by the Clinical Coordinating Center for studies will be linked to the specimen but will be de-identified. Plasma collected for this trial will be frozen and stored at the VCU bio-repository for future research.

7. Statistical Considerations

7.1. Statistical Methods

***CITRIS-ALI Data Analysis Plan*:** We plan to enroll 170 patients in the CITRIS-ALI study (85 per group) to allow for the possibility of approximately 10% dropouts. The projected sample size for this study (n =170) should provide adequate power to detect an absolute 2 point difference on the average SOFA scores between the two study groups (13 vs. 11) with an average SD of 4.6. This will provide an alpha level of 0.05 and a

power of 80%. Effects will be reported with a point estimate and 95% confidence intervals in addition to p-values. We will examine the distributions of all measures and identify possible outliers; outliers will be thoroughly checked for collection or data entry errors before being used in the analysis. All hypotheses will be tested and data analysis will be done using a variety of statistical methods with the most common method expected to be a *Mixed Linear Model* (MLM) for continuous repeated measures. To assess the effect of the treatment on continuous outcome measures that repeat over time the MLM[55,56] will be used to fit a series of repeated measures ANOVA (RMANOVA) models. These models will have one between subject factor (Group; Placebo, Hi-VitC), one within subject factor (Time; Baseline, 12 hours, 24 hours, 36 hours, 48 hours, 72 hours, 96 hours, 7 days) and the interaction between "Group and Time." The Group by Time interaction term will allow us to test the hypothesis that the difference between the treatment groups is the same over time. The MLM that will be used for these analyses differs from the usual general linear model (i.e. ANOVA) in two ways. The MLM allows for the inclusion of both fixed and random effects at the same time and thus allows for the complete analysis of repeated measures designs. Second, observations in the MLM are not required to be independent, as is the case with ANOVA, so that correlated observations that arise from repeated measurements made on the same subjects can be accommodated. For the repeated within subjects measures, a variety of variance-covariance structures will be evaluated to determine which provides the best fit to the observed data. Further, MLMs do not require complete repeated measurements data on all subjects when used to estimate the course of the outcome variable over time. Incomplete or missing data are handled by the model, providing that the missing data are assumed to be "missing at random."[57] While it is expected that the randomization process will prevent any group differences with respect to factors that could impact the outcome measures we can also fit models that include these measures as additional covariates to determine whether any *Group by Time* interactions remain significant. **Early Stopping**: An early stopping determination will be made by the Data Safety Monitoring Board

***Power Estimates Sample Size Calculation*:** The primary goal of this study is to examine the efficacy of vitamin C infusion on organ failure and selected biomarkers. We have chosen the co-primary variables of (1) SOFA score, (2) plasma c-reactive protein and (3) thrombomodulin. The sample size for the proposed study was calculated using observed organ failure data and the biomarker analyses from the phase I clinical trial at VCU. Using data from two biomarkers and the SOFA scores, a power/sample size calculation was conducted. Using the RMANOVA model and assuming an alpha level of 0.05, and using a Holm-Bonferroni correction to accommodate the multiple tests, the following table shows the required sample sizes to detect a significant Group by Time interaction effect at 96 hours.

Power Estimates for a variety of samples sizes, α=0.05 significance level, for multiple primary endpoints using the Holm-Bonferroni Correction

	Empirical Power					
	75/Group	80/Group	85/Group	90/Group	95/Group	100/Group
Co-Primary Variables: SOFA, CRP, TM	77%	77%	80%	80%	82%	83%

Subgroup Analyses from data at enrollment: Shock presence or absence

8. Data Collection and Site Monitoring

8.1. Data Collection

Research coordinators will collect data and enter it directly into the web-based data entry system managed by the Clinical Coordinating Center and record on paper data forms. Data will be transferred to the Clinical Coordinating Center on a prescribed basis through a web-based data entry program.

8.2. Site Monitoring

Remote monitoring will be used and augmented with site visits performed by a contracted monitoring service to ensure that all regulatory requirements are met and to monitor the quality of the data collected. Records of *Institutional Review Board* approvals and patients' charts will be examined on a spot check basis to evaluate the accuracy of the data entered into the database.

9. Risk Assessment

9.1. Risks of Active Study Drug

Intravenous Ascorbic Acid Infusion: High dose ascorbic acid therapy is a powerful anti-oxidant and micronutrient and a safe therapy in normal subjects and in critically ill patients.[58] Muhlhofer and colleagues examined for the presence of adverse effects following intravenous infusion of 7500 mg Ascorbic Acid daily for 6 days in normal subjects (n=6). No abnormalities in laboratory analysis (fasting state; hemoglobin, leukocytes, platelets, sodium, potassium, calcium, chloride, glucose, creatinine, urea, bilirubin, ALT, AST, ggGT, alkaline phosphatase, and prothrombin time were found. Nathens et al infused high dose ascorbic acid (1500 mg three times daily) into critically ill surgical patients for 28 days and found no serious adverse events.[59] Hoffer et al infused dosages as high as 1.5 grams/kg body weight three times weekly into patients with advanced cancer.[60] Adverse events were infrequent consisting of nausea, vomiting, dizziness, and headache. A systematic review conducted by Hans K. Biesalski [61]on the safety of the long term low dose parenteral administration of ascorbic acid in patients on haemodialysis revealed it to be safe with frequent monitoring of oxalate following dialysis. Finally, in the safety study conducted here at VCU testing the safety of infusing high doses of ascorbic acid in patients with sepsis, in which approximately 8 of 24 subjects were receiving dialysis, no adverse events occurred that could be related to the ascorbic acid.

Potential Physical Risks of Ascorbic Acid Infusion: As noted above, the risks associated with ascorbic acid infusion are few. Potential risks include: dry mouth, nausea, vomiting, dizziness, headache.

Potential Psychological, Social, Legal Risks of Ascorbic Acid Infusion: No psychological, social or legal risks are identifiable from an extensive literature search. The recently completed phase I trial: **Vitamin C (Ascorbic Acid) Infusion in Human Sepsis** where up to 16 grams of ascorbic acid was infused daily for 4 days identified no further risk that that identified.

9.2. Risks of Blood Draws

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

9.3. Minimization of Risks

Federal regulations at 45 CFR 46.111(a) (1) requires that risks to subjects are minimized by using procedures which are consistent with sound research design. There are several elements of study design in the present protocol that meets this human subject protection requirement.

Exclusion criteria prohibit participation of patients who might be at increased risk from the effects Vitamin C. Additionally, no adverse events occurred during the pilot study found to be related to the study drug. Finally, vigilant clinical monitoring is standard of care for ICU patients.

9.4. Potential Benefits

Most observational studies suggest a mortality benefit from prior or in-patient Vitamin C use after hospitalization for serious infections. None of the observational trials have reported significant Vitamin C-related toxicity. An animal model of acute lung injury with intravenous LPS and feces induced peritonitis demonstrate significantly less lung injury with Vitamin C, which may result in shortening the time patients require mechanical ventilation.

9.5. Risks versus Benefits

The identifiable risks arising from exposure to intravenous ascorbic acid infusion are low. In our preliminary data, we extensively outlined the potential benefits brought by attenuation of acute lung injury and organ failure associated with bacterial sepsis. Given the low risk associated with ascorbic acid infusion and the potential high likelihood of benefit we assess the risk/benefit ratio to be low (i.e., that benefit far outweighs risk).

10. Human Subjects

Each study participant or a legally authorized representative must sign and date an informed consent form. Institutional review board approval will be required before any subject is entered into the study.

10.1. Selection of Subjects

Screening for patients to be enrolled in the CITRIS-ALI trial will occur in the ICUs at VCU Health System, The Medical College of Wisconsin and sub-site, Aurora St. Luke's Medical Center , The Cleveland Clinic and its sub-site, Fairview Hospital.

10.1.1. Equitable Selection of Subjects

Federal regulations at 45 CFR 46(a)(3) require the equitable selection of subjects. The ICUs will be screened to determine if any patient meets the inclusion and exclusion criteria. Data that have been collected as part of the routine management of the subject will be reviewed to determine eligibility. No protocol-specific tests or procedures will be performed as part of the screening process. If any subjects meet criteria for study enrollment, then the attending physician will be asked for permission to approach the patient or his/her surrogate for informed consent. Justifications of exclusion criteria are given in Section 4.3. These exclusion criteria neither unjustly exclude classes of individuals from participation in the research nor unjustly include classes of individuals from participation in the research. Hence, the recruitment of subjects conforms to the principle of distributive justice.

10.1.2. Vulnerable Subjects

The present research aims to investigate the safety and efficacy of a type of treatment for patients with ALI and ARDS secondary to severe sepsis. No vulnerable subjects will be entered into this phase II trial.

10.2. Informed Consent

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. The investigator is responsible for ensuring that the patient or patient's legal representative understands the risks and benefits of participating in the study,

and answering any questions the patient may have throughout the study and sharing any new information in a timely manner that may be relevant to the patient's or the legal representative'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.

10.3. Identification of Surrogates

Many of the patients approached for participation in this research protocol will have limitations of decision-making abilities due to their critical illness. Hence, most patients will not be able to provide informed consent. Accordingly, informed consent will be sought from the potential subject's legally authorized representative.

Regarding proxy consent, the existing federal research regulations ('the Common Rule') state at 45 CFR 46.116 that: ***"no investigator may involve a human being as a subject in research…unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative"*;** and defines at 45 CFR 46 102 (c) a legally authorized representative (LAR) as: ***"an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject's participation in the procedures(s) involved in the research."*** OHRP defined examples of "applicable law" as being state statutes, regulations, case law, or formal opinion of a State Attorney General that addresses the issue of surrogate consent to medical procedures. Such "applicable law" could then be considered as empowering the surrogate to provide consent for subject participation in the research. Interpretation of "applicable law" is therefore state specific and hence, will be left to the discretion of the individual IRBs of the respective clinical centers involved in the CITRIS-ALI trial.

According to a previous President's Bioethics Committee (National Bioethics Advisory Committee), an investigator should accept as an LAR…***a relative or friend of the potential subject who is recognized as an LAR for purposes of clinical decision making under the law of the state where the research takes place***.[62] Finally, OHRP has opined in their determination letters that a surrogate could serve as a LAR for research decision making if such an individual is authorized under applicable state law to provide consent for the "procedures" involved in the research study.[63]

10.4. Justification of Surrogate Consent

According to the Belmont Report, respect for persons incorporates at least two ethical convictions; first, that individuals should be treated as autonomous agents, and second, that persons with diminished autonomy are entitled to protection. One method that serves to protect subjects is restrictions on the participation of subjects in research that presents more than minimal risks. Commentators and Research Ethics Commission have held the view that it is permissible to include incapable subjects in research that involves more than minimal risk as long as there is the potential for beneficial effects and if the research presents a balance of risks and expected direct benefits ***similar*** to that available in the clinical setting.[64] Several U.S. task forces have deemed it is permissible to include incapable subjects in research. For example, the American College of Physicians' document allows surrogates to consent to research involving incapable subjects only "*if the net additional risks of participation are not substantially greater than the risks of*

standard treatment."[65] Finally, the National Bioethics Advisory Committee (NBAC) stated "*that an IRB may approve a protocol that presents greater than minimal risk but offers the prospect of direct medical benefits to the subject, provided that…the potential subject's LAR gives permission…*"

Consistent with the above ethical sensibilities regarding the participation of decisionally incapable subjects in research and the previous assessment of risks and benefits in the previous section, the present trial presents a balance of risks and potential direct benefits that is ***similar*** to that available in the clinical setting, with the exception of the additional blood draws.

10.5. Additional Safeguards for Vulnerable Subjects

The present research will not involve subjects who might be vulnerable to coercion or undue influence.

10.6. Confidentiality

Federal regulations at 45 CFR 46 111 (a) (7) requires that when appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of data. To maintain confidentiality, all laboratory specimens, evaluation forms, and reports will be identified only by a coded number. The coded number will be generated at random by a computer, and only the study investigators will have access to the codes. All records will be kept in a locked, password protected computer. All computer entry and networking programs will be done with coded numbers only. All paper case report forms will be maintained in a locked cabinet inside a locked office. Clinical information will not be released without the written permission of the patient, except as necessary for monitoring by the National Heart, Lung, and Blood Institute, the Federal Drug Administration or other authorized Federal Agencies.

11. Adverse Event Reporting/Safety Reporting

Investigators will determine daily if any clinical adverse experiences occur during the period from informed consent through study hour 168 and will be followed up through resolution, resolved with sequelae, unresolvable or death.

It is expected that Diseases/Illnesses/Symptoms associated with the SEPSIS ALI study population will occur in the study population, independent of investigation product exposure. These associated diseases/illnesses/symptoms will be considered as part of the study inclusion processes and/or study assessments and as such will not be considered 'reportable' Adverse Events (AE)/Serious Adverse Events (SAE) unless the Investigator has a reasonable doubt regarding the relatedness of the event to the investigational product.

The investigator will evaluate any changes in laboratory values and physical signs and will determine if the change is clinically important and different from what is expected in the course of treatment of patients with ALI. If clinically important and unexpected adverse experiences occur, they will be recorded on the adverse event case report form.

The following will be considered reportable adverse events:

For this trial, a *reportable adverse event* is defined as:

1. Any clinically important untoward medical occurrence in a patient receiving study drug or

undergoing study procedures which is different from what is expected in the clinical course of a patient with severe sepsis associated ALI, or,

2. Any clinically important, untoward medical occurrence that is thought to be associated with the study drug or procedures, regardless of the "expectedness" of the event for the course of a patient with severe sepsis associated ALI.
3. Investigators will report all *serious, unexpected, AND study-related* adverse events from the time of informed consent through study hour 168 that are considered to be harmful and unintended responses to the investigational product and/or study related procedures in the participants' case report forms. 'Responses to investigational product' means that the causal relationship between an investigational product and an adverse event cannot be ruled out.

Expected Events For ALI considered unreportable: *refer to Appendix F Table*

1. Defined as: Untoward clinical occurrences perceived by the investigator to occur with reasonable frequency in the day to day care of patients with ALI treated in an intensive care unit with mechanical ventilation.
2. Examples of untoward clinical occurrences that are expected in the course of ALI include: 1) transient hypoxemia, 2) agitation, 3) delirium, 4) nosocomial infections, 5) skin breakdown, and 6) gastrointestinal bleeding. Such events, which are often the focus of prevention efforts as part of usual ICU care, will not be considered reportable adverse events unless the event is considered by the investigator to be associated with the study drug or procedures, or unexpectedly severe or frequent for an individual patient with ALI. Examples of unexpectedly frequent untoward clinical occurrences would be *repeated* episodes of unexplained hypoxemia. This would be in contrast to an isolated episode of transient hypoxemia (e.g., $Sp0_2$ ~85%), related to positioning or suctioning. This latter event would not be considered unexpected by nature, severity or frequency.
3. Adverse events occurring from the time of informed consent through study hour 168 or until discharged from the hospital, withdrawal from the study or death, will not be considered 'reportable' Adverse Events (AE)/Serious Adverse Events (SAE) unless the Investigator has a reasonable doubt regarding the relatedness of the event to the investigational product.

The following will be reported as adverse events:

Investigators will report all unanticipated problems that **involve risk or harm to a research participant** AND **was not anticipated or foreseen (e.g., not described in the consent form)** AND **is probably or definitely related to or caused by the research**, as defined in Appendix F, to the Data Coordinating Center by phone and email within 24 hours of becoming aware of event. The Institutional Review Board for the lead site will be notified within 5 business days of receiving notice of the unanticipated problem. Participating sites shall report to their Institutional Review Board in accordance with their institution's rules and regulations.

The Data Coordinating Center (VCU) will report all, unanticipated problems, defined as problems that **involve risk or harm to a research participant** AND **was not anticipated or foreseen (e.g., not described in the consent form)** AND **is probably or definitely related to or caused by the research**, to the DSMB within 7 calendar days of the CCC being notified of the event. The Data Coordinating Center will distribute the written summaries of the DSMB's periodic reviews to participating centers.

The Data Coordinating Center will also determine if the serious adverse event is unexpected for Vitamin C. Unexpected for Vitamin C is defined as any event not listed in the Vitamin C package insert. If the Data Coordinating Center determines that any serious and study-related adverse event is unexpected for Vitamin C, the FDA will be notified within 7 calendar days. Such events may also meet the definition of *Unanticipated Problems* as described below.

Investigators must report *Unanticipated Problems*, regardless of severity, associated with the study drug or study procedures within 24 hours. An unanticipated problem is defined as follows:

Unanticipated Problem (UP): any incident, experience, or outcome that meets all of the following criteria will be reported from the time of consent through study hour 168 until resolved, withdrawn from the study, death occurs or lost to follow up:

- Unexpected, in terms of nature, severity, or frequency, given the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and the characteristics of the subject population being studied;
- Related or possibly related to participation in the research, in this guidance document, 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;
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

1. **Safety Reporting:**

Investigator safety reports are prepared for suspected unanticipated serious adverse reactions according to local regulatory requirements and are forwarded to investigators as necessary. An investigator who receives an investigator safety report describing an SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from the Clinical Coordinating Center will file it and will notify the IRB/IEC, if appropriate, according to local requirements.

12. APPENDICES

APPENDIX A: Guidelines for evidence of infection

1. **Infections of the thorax:**
    a) Chest x-ray or CT scan showing a new or progressive infiltrate, consolidation, cavitation, collection, or pleural effusion, and a clinical presentation consistent with pneumonia or empyema
    b) Pneumonia can be defined as the presence of new infiltrate (s), absence of a noninfectious explanation and either signs of SIRS as per protocol or purulent sputum production with an identifiable pathogen.
    c) Aspiration Pneumonitis in the acute phase is not considered an infection.
    d) However, if SIRS persists > 24 hours after aspiration, then an infectious etiology can be presumed.
2. **Abdominal infection:**
    a) Perforated viscus or ischemic bowel with either localized peritonitis
    b) Peritoneal fluid with > 250 PMNs
    c) Clinical signs of cholangitis or appendicitis
    d) Clostridium difficile toxin positive with evidence of colon dilation
    e) Suspicion of peritonitis by clinical examination only
3. **Skin or soft tissue infection:** Acute onset infection of the skin, such as erysipelas, or infection involving deeper soft tissue
4. **Bacterial meningitis:** cerebrospinal fluid analyses if available and a clinical presentation consistent with bacterial meningitis
5. **Urinary Tract:**
    a) Positive test for granulocyte esterase or nitrate in urine, or a positive culture (defined as $>10^5$ CFU/mL)
    b) Urinalysis with increased WBC count or positive Gram stain
6. **Central Line infections:**
    a) Catheter-related bloodstream infections (CR-BSIs) are defined as bacteremia/fungemia in a patient with an intravascular catheter with at least one positive blood culture obtained from a peripheral vein, clinical manifestations of infection (i.e., fever, chills, and/or hypotension), and no apparent source for the bloodstream infection except the catheter. The catheter must be in place for at least 48 hours prior to development of the bloodstream infection.

7. **Sinusitis**
    a) Air fluid levels in sinus seen on CT scan

** Use of antibiotics at time of consent (provided the antibiotics are not for prophylaxis) is considered evidence of suspected infection. Examples of prophylactic antibiotics include: pre-surgical incision, antibiotic for the prevention of pneumocystis jiroveci (aka carinii), herpes simplex, cytomegaloviryus, and latent mycobacterial disease.

The following are not considered evidence of infection:

a) Fever of unknown origin
b) Blood cultures that are considered positive only because of the isolation of a likely contaminant organism
c) Postoperative hypotension within 24 hours of incision and/or fever without a verified infectious focus.
d) Leukocytosis alone in the presence of steroid usage is insufficient evidence of infection.

e) Leukocytosis alone in the presence of connective tissue disorder is insufficient evidence of infection.

APPENDIX B: Pleiotropic Effects of Vitamin C

1. **Cell culture/in vitro studies**
   - Reduced human neutrophil adhesion to endothelial cells[66]
   - Protects neutrophils against intracellular effects of superoxide generation.[67]
   - Protects monocytes against oxidative damage[68]
   - Reduced PMA induction of NF-κB
   - Protects against oxidized-LDL-induced expression of MCP-1 in cultured human umbilical vein endothelial cells[69]
   - Modulates the inhibition of platelet aggregation by neutrophils[70]
   - Inhibits expression of platelet expression of CD40 ligand which promotes thrombosis[71]
   - Inhibits NADPH oxidase subunit p47phox expression in microvascular endothelium[72]
   - Influences dendritic cell function.[73]
   - Effects of vitamin C on intra cytoplasmic cytokine production in human whole blood monocytes and lymphocytes. exposed to LPS or immune complexes[74]
   - Inhibition of the induction of inducible nitric oxide sythetase (iNOS) and TNF-α, IL-1β and IL-6 in astrocytes, microglia and macrophages stimulated with LPS or cytokines
   - Vitamin C inhibits NO-induced stabilization of HIF-1alpha in HUVECs[75]
   - Cobalt-induced oxidant stress in cultured endothelial cells: prevention by ascorbate in relation to HIF-1alpha.[76]

2. **Intact animal studies**
   - Attenuates LPS induced acute lung injury.[77]
   - Attenuates lung injury in a cecal ligation and puncture model of peritonitis.[78]
   - Corrects capillary blood flow in septic skeletal musculature.[79]
   - Inhibits iNOS expression in septic vasculature.[80]
   - Attenuates iNOS expression in IFN gamma-stimulated rat skeletal muscle endothelial cells[81]
   - Attenuates hepatic fibrosis by upregulating peroxisome proliferators-activated receptor-gamma[82]
   - Inhibits both flow- and agonist-induced EDHF in the rat mesentery[83,84]
   - Multiple molecular transporters responsible for movement of ascorbate intracellular[85]
   - Attenuates peroxidative damage and tissue edema in ischemia/reperfused gut.[86]
   - Ascorbate supplementation significantly decreases plasma IL-6 levels in an animal model of hemorrhagic shock.[87]
   - Vitamin C deficiency causes the collagen-disassembly disease scurvy
   - Ascorbic acid prevents testosterone-induced hyperplasia of rat prostate by down-regulating HIF-1alpha[88]
   - Plasma AA maintains the stability of “acellular Hb” susceptible to oxidation[89]
   - Endogenous ascorbate on oxidation, oxygenation, and toxicokinetics of cell-free modified hemoglobin after exchange transfusion in rat and guinea [90]

Human studies

   - Normalizes monocyte adhesion to endothelium in vitamin C deficient subjects. [91]
   - Supplementation in chronic hemodialysis patients reduce lymphocyte 8-OHdG levels and intracellular ROS production [92]
   - Increase in muscle blood flow during dynamic exercise with acute AA administration in older adult humans[93]
   - Intravenous ascorbate improves outcomes during percutaneous myocardial intervention[94]
   - Plasma vitamin C level positively associated with serum pre-albumin levels and negatively associated with high sensitivity C-Reactive Protein levels in patients with chronic renal failure.[95,96]

- Low plasma ascorbate levels are associated with enhanced proinflammatory responses and impaired vascular function in lean and obese men.[97]
- Reduced inflammatory tissue damage in patients subjected to cardiac surgery with extracorporeal circulation[98]
- Ascorbate promotes iron utilization for erythropoiesis in patients with chronic renal failure[99]
- High dose vitamin C infusion into surgically critically ill daily for 28 days attenuated the incidence of acute lung injuy/ARDS.[100]

APPENDIX C: Ventilator Procedures

C.1. Ventilator Management

A modified, simplified version of the ARDS Network lung protective lower tidal volume strategy will be used in this trial. This strategy, which was associated with low mortality rates in three previous ARDS Network trials (ARMA, ALVEOLI, and FACTT), will ensure that study subjects receive the beneficial effects of lung protection while participating in this trial.[101] [102] The PI/PDs (Drs. Fowler, Truwit, Hite, Martin) and professionals at the CITRIS-ALI consortium medical centers have significant experience with the application of ARDS Network ventilation protocols.

1. Any mode of ventilation capable of delivering the prescribed tidal volume (V_T, 6ml/kg predicted body weight, +/- 2ml/kg) may be used, provided the V_T target is monitored and adjusted appropriately. If airway pressure release ventilation (APRV) is used, tidal volume is defined as the sum of the volume that results from the ventilator pressure- release and an estimation of the average spontaneous V_T.
2. V_T Goal: 6 ml / kg predicted body weight.
3. Predicted body weight (PBW) is calculated from age, gender, and height (heel to crown)
    a. according to the following equations:
    b. Males: PBW (kg) = 50 + 2.3 [height (inches) – 60]
    c. Females: PBW (kg) = 45.5 + 2.3 [height (inches) – 60]
4. Measure and record inspiratory plateau pressure (Pplat) according to ICU routine (at least every four hours and after changes in V_T and PEEP recommended)
5. If Pplat > 30 cm H_2O, reduce V_T to 5 ml/kg and then to 4 ml/kg PBW if necessary to decrease Pplat to ≤ 30 cm H_2O.
6. If V_T < 6 ml/kg PBW and Pplat < 25 cm H_2O, raise V_T by 1 ml/kg PBW to a maximum of 6 ml/kg.
7. If "severe dyspnea" (more than 3 double breaths per minute or airway pressure remains at or below PEEP level during inspiration), then raise V_T to 7 or 8 ml/kg PBW if Pplat remains below 30 cm H_2O. If Pplat exceeds 30 cm H_2O with V_T of 7 or 8 ml/kg PBW, then revert to lower V_T and consider more sedation.
8. If pH < 7.15, V_T may be raised and Pplat limit suspended (not required).
9. Oxygenation target: **[55 mm Hg < PaO_2 < 80 mm Hg]** or **[88% < SpO_2 < 95%]**. When both PaO_2 and SpO_2 are available simultaneously, the PaO_2 criterion will take precedence.
10. Minimum PEEP = 5 cm H_2O
11. Adjust F_iO_2 or PEEP upward within 5 minutes if there are consistent measurements below the oxygenation target range
12. Adjust F_iO_2 or PEEP downward within 30 minutes if there are consistent measurements above the oxygenation target range.
13. There are no requirements for maintaining a specific PEEP to F_iO_2 ratio. The lower PEEP/higher F_iO_2 table represents a consensus approach developed by ARDS Network investigators in 1995. The higher PEEP/lower F_iO_2 table (ALVEOLI) yielded equivalent results in a randomized trial and would be acceptable and perhaps preferable in patients who appear to respond with a substantial increase in arterial oxygenation in the transition from lower to higher PEEP.

Lower PEEP/Higher F_iO_2 Treatment Group

F_iO_2	.30	.40	.40	.50	.50	.60	.70	.70	.70	.80	.90	.90	.90	1.0
PEEP	5	5	8	8	10	10	10	12	14	14	14	16	18	18-24

Higher PEEP/Lower F_iO_2 Study Group

F_iO_2	.30	.30	.30	.30	.30	.40	.40	.50	.50	.50 – .80	.80	.90	1.0	1.0
PEEP	5	8	10	12	14	14	16	16	18	20	22	22	22	24

Note: Levels of PEEP in these F_iO_2/ PEEP tables represent levels set on the ventilator, not levels of total-PEEP, auto-PEEP, or intrinsic-PEEP.

14. No specific rules for respiratory rate. It is recommended that the respiratory rate be increased in increments to a maximum set rate of 35 if pH < 7.30.
15. No specific rules about I:E ratio. It is recommended that duration of Inspiration be ≤ duration of Expiration.
16. Bicarbonate is allowed (neither encouraged nor discouraged) if pH < 7.30.
17. Changes in more than one ventilator setting driven by measurements of PaO2, pH, and Pplat may be performed simultaneously, if necessary.

C.2. Weaning

Commencement of Weaning (applicable to patients ventilated invasively or non-invasively)
Patients will be assessed for the following weaning readiness criteria each day between 0600 and 1000. If a patient procedure, test, or other extenuating circumstance prevents assessment for these criteria between 06:00 and 10:00, then the assessment and initiation of subsequent weaning procedures may be delayed for up to six hours.

1. At least 12 hours since enrollment in the trial
2. F_iO_2 ≤ 0.40 and PEEP ≤ 8 cm H_2O **or** F_iO_2 ≤ 0.50 and PEEP = 5 cm H_2O
3. Values of both PEEP and F_iO_2 **≤** values from previous day
4. Not receiving neuromuscular blocking agents and without neuromuscular blockade
5. Patient exhibiting inspiratory efforts. If no efforts are evident at baseline, ventilator set rate will be decreased to 50% of baseline level for up to 5 minutes to detect inspiratory efforts.
6. Systolic arterial pressure **≥** 90 mm Hg without vasopressor support (≤ 5 mcg/kg/min dopamine will not be considered a vasopressor)

Spontaneous Breathing Trial Procedure and Assessment for Unassisted Breathing

If criteria 1-6 above are met, then initiate a trial of up to 120 minutes of spontaneous breathing with F_IO_2 < 0.5 using any of the following approaches:

1. Pressure support (PS) < 5 cm H_2O, PEEP < 5 cm H_2O
2. CPAP < 5 cm H_2O
3. T-piece
4. Tracheostomy collar (mask)

The clinical team may decide to change mode during spontaneous breathing (PS = 5, CPAP, tracheostomy mask, or T-piece) at any time during the spontaneous breathing trial.

Monitor for tolerance using the following:

1. SpO_2 ≥ 90% and / or PaO_2 ≥ 60 mm Hg
2. Mean spontaneous tidal volume ≥ 4 ml/kg PBW (if measured)
3. Respiratory Rate ≤ 35 / min
4. pH ≥ 7.30 (if measured)
5. No respiratory distress (defined as 2 or more of the following):
    a. Heart rate ≥ 120% of the 0600 rate (≤ 5 min at > 120% may be tolerated)
    b. Marked use of accessory muscles
    c. Abdominal paradox
    d. Diaphoresis
    e. Marked subjective dyspnea

If any of the goals a-e are not met, revert to previous ventilator settings or to PS greater than or equal to 10 cm H_2O with Positive End-expiratory Pressure and F_iO_2 = previous settings and reassess for weaning the next morning. The patient will be reassessed for weaning (Section C2) the following day.

Decision to remove ventilatory support:

If tolerance criteria for spontaneous breathing trial (a-e above) are met for at least 30 minutes, the clinical team may decide to discontinue mechanical ventilation. However, the spontaneous breathing trial can continue for up to120 minutes if tolerance remains in question.

C.3. Definition of Unassisted Breathing

1. Spontaneously breathing with face mask, nasal prong oxygen, or room air, OR
2. T-tube breathing, OR
3. Tracheostomy collar (mask) breathing, OR
4. CPAP ≤ 5 without PS or IMV assistance
5. Use of CPAP or BIPAP solely for sleep apnea management

C.4. Definition of Extubation

1. Removal of an oral or nasotracheal tube
2. If a patient receives a tracheostomy, the time of extubation is defined as the time when the patient achieves unassisted breathing as defined in section C.3

C.5. Completion of Ventilator Procedures

Patients will be considered to have completed the study ventilator procedures if any of the following conditions occur:

1. Death
2. Hospital discharge
3. Alive 28 days after enrollment

If a patient requires positive pressure ventilation after a period of unassisted breathing, the study ventilator procedures will resume unless the patient was discharged from the hospital or > 28 days elapsed since enrollment.

C.6. Removal from the Ventilator Management Protocol

Patients may be removed from the 6 ml/kg PBW tidal volume ventilation requirement if they develop

neurologic conditions where hypercapnia would be contraindicated (e.g., intracranial bleeding, GCS < 8, cerebral edema, mass effect [midline shift on CT scan], papilledema, intracranial pressure monitoring, fixed pupils).

APPENDIX D: FACTT LITE Conservative Fluid Management Approach

If patient does not have a MAP > 60mmHg and has not been off vasopressors for > 12 hours, then patient does not meet criteria for any actions prescribed in the Fluid Management Approach. Document as "Not Clinically Indicated".

This fluid protocol captures the primary positive outcome of the FACTT trial on increasing ventilator free days. If clinically possible, for patients with a CVC, this protocol should be initiated within four hours of randomization in enrolled patients, and continued until UAB or study day 7, whichever occurs first.

- Discontinue maintenance fluids.
- Continue medications and nutrition.
- Manage electrolytes and blood products per usual practice.
- For shock, use any combination of fluid boluses[#] and vasopressor(s) to achieve MAP ≥ 60 mmHg as fast as possible. Wean vasopressors as quickly as tolerated beginning four hours after blood pressure has stabilized.
- Withhold diuretic therapy in renal failure [§] and until 12 hours after last fluid bolus or vasopressor given.

CVP (recommend)	PAOP (optional)	MAP ≥ 60 mm Hg AND off vasopressors for ≥ 12 hours	
		Average urine output < 0.5 ml/kg/hr	Average urine output ≥ 0.5 ml/kg/hr
>8	> 12	Furosemide* Reassess in 1 hour	Furosemide* Reassess in 4 hours
4-8	8-12	Give fluid bolus as fast as possible[#] Reassess in 1 hour	
< 4	< 8		No intervention Reassess in 4 hours

§ Renal failure is defined as dialysis dependence, oliguria with serum creatinine > 3mg/dl, or oliguria with serum creatinine 0-3 with urinary indices indicative of acute renal failure.

[#] Recommended fluid bolus= 15 mL / kg crystalloid (round to nearest 250 mL) or 1 Unit packed red cells or 25 grams albumin

*Recommended Furosemide dosing = begin with 20 mg bolus or 3 mg / hr infusion or last known effective dose. Double each subsequent dose until goal achieved (oliguria reversal or intravascular pressure target) or maximum infusion rate of 24 mg / hr or 160 mg bolus reached. Do not exceed 620 mg / day. Also, if patient has heart failure, consider treatment with dobutamine.

APPENDIX E: Time Events Schedule

Assessments	Hour 0	Q 24 hrs 1st 7 days or in ICU	Hour 48	Hour 96	Hour 168	Day 28	Day 60
VS: BP, HR, MAP, RR, Temp., O2 sats, CVP, Glasgow Coma Scale	X		X	X	X		
Body Weight	A		A	A			
Suspected or known site of sepsis	A		A	A	A	A	A
I/Os Total and Urine only		X					
Assessment of Acute/Chronic Renal Failure and Use of Dialysis	X		X	X	X	X	
Calculate SOFA Score (post study by biostatistician)	X		X	X	X		
Calculate VE40 Score (post study by biostatistician)	A		A	A	A		
Calculate Oxygenation Index (post study by biostatistician)	A		A	A	A		
Ventilator Data:							
Tv, FiO2, PEEP, Plateau Pressure, Peak Inspiratory Pressure, Mean Airway Pressure, Minute Ventilation	A		A	A	A		
Labs:							
Arterial Blood Gasses, Na+, K+, BUN, Cr, WBC, Hgb, Hct, Platelets, PT/INR	X		X	X	X		
Bilirubin Total	X		X	X	X		
Vasopressors or Inotropes:							
Epi, Nor-epi, Phenylephrine, Vasopressin, Dopamine	A		A	A	A		
Concomitant Medications: Methylprednisone, Hydrocortisone	X		X	X	X		
AE/SAE Assessments	X		X	X	X		
Blood for Biomarkers	X		X	X	X		
Ventilator Free Days						X	
All Cause Mortality						X	
ICU Free Days						X	
Hospital Free Days							X
Glucose Monitoring	I	I	I	I	I		

X = Required
I= As Needed (See Section 3.3.2)
A=When available

APPENDIX F: Adverse Events

Procedures for Reporting Adverse Events

Assuring patient safety is an essential component of this protocol. Each participating investigator has primary responsibility for the safety of the individual participants under his or her care. The Principal Investigator will evaluate all adverse events. The Study Coordinator must view patient records for possible adverse events throughout the study period.

AE/SAEs that meet the definition of reportable events (refer to Appendix F Table 1: Anticipated AEs) or as determined by the investigator, will be followed from the time of consent through study hour 168 until resolved, resolved with sequelae, unresolvable, or death.

SAEs will be collected over the same time period as stated above for AEs. However, any AEs/SAEs assessed as related to study participation (e.g. Disease under study) will be recorded from the time of consenting up to and including study hour 168, discharge from the hospital, withdrawal, lost to follow up, or death.

Events that **do not** meet the definition of an AE include:

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.
- The disease/disorder being studied, or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen;
- "Lack of efficacy" or "failure of expected pharmacological action" would not be reported as an AE or SAE. However, the signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they meet the definition of an AE or SAE.

If an event does not meet the definition of an AE per Section 11, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease, etc).

SAE Follow-up language:

All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up.

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by the Lead Site to explain as fully as possible the nature and/or causality of the AE or SAE. The investigator is obligated to assist. This may include additional

laboratory tests or procedures, or consultation with other health care professionals. If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide the Lead Site with a copy of any autopsy reports.

New or updated information will be recorded on the SAE report form. The investigator will submit any updated SAE form to the Lead Site within the designated reporting time frames.

Once the investigator determines that an event meets the protocol definition of an SAE, the SAE will be reported to the Lead Site **within 24 hours**. Any follow-up information on a previously reported SAE will also be reported to the Lead Site within 24 hours.

The investigator will always provide an assessment of causality at the time of the initial report as described in Section 11.

The primary mechanism for reporting SAEs to the Lead Site will be through REDCap and facsimile with an accompanying email. If the electronic system is unavailable for greater than 24 hours, the site will fax and email. Then the site will enter the serious adverse event data into REDCap as soon as it becomes available.

After the study is completed at a given site, REDCap will be taken off-line to prevent the entry of new data or changes to existing data. If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after REDCap has been taken off-line, the site can report this information on a paper SAE form via email.

Facsimile transmission of the SAE form is the preferred method to transmit this information to the Lead Site for SAE receipt. In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable, with a copy of the SAE data collection tool sent by overnight mail. Initial notification via the telephone does not replace the need for the investigator to complete and sign the SAE data collection tool within the designated reporting time frames.

1. *Serious, Expected, AND Study-Related Adverse Events*: adverse events occurring from the time of informed consent through study hour 168 or until discharged from the hospital, withdrawal from the study, or death will not be considered 'reportable' Adverse Events (AE)/Serious Adverse Events (SAE) unless the Investigator has a reasonable doubt regarding the relatedness of the event to the investigational product (refer to Table: Anticipated AE/SAEs for the SEPSIS ALI Study Population).

2. *Serious, Unexpected, AND Study-Related Adverse Events:* Investigators will report all *serious, unexpected, AND study-related* adverse events from the time of informed consent through study hour 168, to the Clinical Coordinating Center within 24 hours by email. The local Institutional Review Board must also be notified in a timely manner. The investigator will then submit a detailed written report to the Clinical Coordinating Center and the local Institutional Review Board no later than 5 calendar days after the investigator discovers the event.

3. Definitions of Adverse Events
    a. A *serious* adverse event is any event that is fatal or immediately life threatening, is permanently disabling, or severely incapacitating, or requires or prolongs inpatient hospitalization. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious adverse events when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Life-threatening means that the patient was, in the view of the investigator, at immediate risk of death from the

reaction as it occurred. This definition does not include a reaction that, had it occurred in a more serious form, might have caused death. Assessment of the cause of the event has no bearing on the assessment of the event's severity.

b. An *unexpected* event is any experience not identified by the type, severity, or frequency in the current study protocol or an event that is unexpected in the course of treatment for ALI or ARDS.

c. Adverse events will be considered to be study-related if the event follows a reasonable temporal sequence from a study procedure and could readily have been produced by the study procedure.

d. Organ failures or death related to ALI or 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*.

4. Assigning Causality

a. The investigator is obligated to assess the relationship between investigational product and the occurrence of each AE/SAE. A "reasonable possibility" is meant to convey that there are facts/evidence or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. The investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the sequential relationship of the event to the investigational product will be considered and investigated. The investigator will also consult the Investigator Brochure (IB) and/or Product Information, for marketed products, in the determination of his/her assessment.

b. There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to VCU. However, **it is very important that the investigator always make an assessment of causality for every event prior to the initial transmission of the SAE data to VCU.** The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE report form.

- **Unrelated:**
  - Event occurred before dosing;
  - Event or concomitant illness due to factors other than drug or study procedure;
- **Possibly:**
  - Reasonable sequential relationship with study procedure or drug treatment;
  - Event could be explained by patient's clinical state or other factors
- **Probably:**
  - Reasonable sequential relationship with study procedure or drug treatment;
  - Likely to be a known reaction to study agent or chemical group, or predicted by known pharmacology;
  - Event cannot easily be explained by patient's clinical state or other factors.
- **Definitely:**
  - Distinct sequential relationship with study procedure or drug treatment;
  - Known reaction to study agent or chemical group, or predicted by known pharmacology;
  - Event cannot be explained by patient's clinical state or other factors.

ANTICIPATED AE/SAEs for the SEPSIS ALI Study Population

The following is a list of anticipated Diseases/Illnesses associated with the SEPSIS ALI study population and are expected to occur in the study population, independent of investigation product exposure; will be captured as part of the study inclusion processes and/or study assessments and as such will not be considered

'reportable' Adverse Events (AE)/Serious Adverse Events (SAE) unless the Investigator has a reasonable doubt regarding the relatedness of the event to the investigational product, from the time of informed consent through Study hour 168 or until discharged from the hospital, withdrawn from the study or death.

AE/SAEs that meet the definition of reportable events or as determined by the investigator, will be followed from the time of consent through study hour 168 until resolved, resolved with sequelae or considered unresolvable.

NOTE: this list is meant to be as comprehensive as possible for expected events for the Disease under study and may not include all events. The Investigator responsibilities for reporting of AE/SAEs still apply, regardless of the table below and should be reported if the Investigator has a reasonable doubt regarding the relatedness of the event to the investigational product.

Table 1:

Expected Disease(s) Under Study	**Associated Symptoms and Labs**
Infection	(e.g., thorax, urinary tract, abdomen, skin, sinuses, bacterial meningitis, central venous catheters, and central nervous system, **see Appendix A**) ** Use of antibiotics at time of consent (provided the antibiotics are not for prophylaxis) is considered evidence of suspected infection. Examples of prophylactic antibiotics include: pre-surgical incision, antibiotic for the prevention of pneumocystis jiroveci (aka carinii), herpes simplex, cytomegalovirus, and latent mycobacterial disease.
Acute Lung Injury (ALI)	**Fever:** >38ºC (any route) or hypothermia: <36ºC (core temp only), **Tachycardia:** heart rate > 90 beats/min or receiving medications that slow heart rate or paced rhythm, **Leukocytosis:** >12,000 WBC/μL or **leukopenia:** <4,000 WBC/μL or >10% band forms. **Respiratory rate** > 20 breaths per minute or **PaCO2** < 32 or invasive mechanical ventilation.
Adult Respiratory Distress Syndrome (ARDS)	**Lung injury of acute onset**, within 1 week of an apparent clinical insult and with progression of respiratory symptoms; **Bilateral opacities on chest imaging** not explained by other pulmonary pathology (e.g. pleural effusions, lung collapse, or nodules); **Respiratory failure** not explained by heart failure or volume overload; **Decreased arterial PaO2/FiO2 ratio** ≤ 300 mm Hg; **Minimum PEEP** of 5 cmH_2O
Systemic Inflammatory Responses (SIRS)	**Fever** > 38°C (any route) or hypothermia: < 36°C (core temp. only) **Tachycardia**: heart rate > 90 beats/min or receiving medications that slow heart rate or paced rhythm **Respiratory Rate** > 20 breaths per minute or PaC02 < 32 or invasive mechanical ventilation **Leukocytosis**: > 12,000 WBC /μL or leukopenia: <4,000 WBC/μL or >10% band forms
Sepsis associated System Organ Failure (SOF) The current trial will be enrolling patients with sepsis associated acute lung injury. We therefore expect that many of these patients will have some degree of organ dysfunction.	**Sepsis associated hypotension** (systolic blood pressure (SBP) < 90 mm Hg or an SBP decrease > 40 mm Hg unexplained by other causes or use of vasopressors for blood pressure support (epinephrine, norepinephrine, dopamine =/> 5mcg, phenylephrine, vasopressin); **Arterial hypoxemia** (PaO_2/FiO_2 < 300) or supplemental O2 > 6LPM. **Lactate** > upper limits of normal laboratory results **Urine output** < 0.5 ml/kg/hour for > two hours despite adequate fluid resuscitation **Platelet count** < 100,000 per mcL

	Coagulopathy (INR > 1.5) **Bilirubin** > 2 mg/dL **Glasgow Coma Scale** < 11 or a positive CAM ICU score
Biomarkers of Inflammation, Vascular Injury and Alveolar epithelial injury	Increases in C-reactive protein (CRP), procalcitonin (PCT), thrombomodulin (TM) alveolar epithelial injury (Receptor for Advanced Glycation Products)
Lactic Acidosis	Patients with sepsis are at high risk of metabolic acidosis (including lactic acidosis). To prevent the possibility of metabolic acidosis due to drug administration, the study drug is formulated to a neutral pH of 7.4. Therefore, we do not anticipate the need for additional monitoring of acid/base balance beyond standard-of-care provided at each institution. Any observed abnormalities will be evaluated according to standard-of-care practice and documented in the research record.
Use of Dialysis	In the presence of Acute or Chronic renal failure.
Septic Shock	Fluid management during shock will not be prescribed per study protocol. In subjects who are not in shock, a conservative fluid management approach will be administered, if possible. (refer to Appendix D, Table 5).
Use of Ventilator	As per protocol (refer to Section 5.5 and Appendix C)
Plasma Ascorbate levels	Septic patients exhibit subnormal plasma ascorbate levels. Phase I studies performed at Virginia Commonwealth University (VCU) show mean ascorbate levels of 17.5 µM (normal human ascorbate levels 60 to 70 µM). The day 2 – 7 plasma ascorbate levels are expected to be between 500 to 1000 µM.
Death	Organ failures or death related to ALI or 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. (refer to Appendix F).*
NOTE: All AE/SAEs considered "Reportable" will be captured on the adverse event log and reported to the sponsor.	

APPENDIX G: Modified SOFA Score Calculator

Value	0	1	2	3	4	Score
Respiratory **PaO_2/FiO_2**	**>400**	**≤400**	**≤300**	**≤200 with respiratory support**	**≤100 with respiratory support**	
Coagulation **Platelets**	**>150**	**≤150**	**≤100**	**≤50**	**≤20**	
GI **T Bilirubin**	**<1.2**	**1.2-1.9**	**2.0-5.9**	**6.0-11.9**	**□12**	
Cardio-Vascular	**No Hypo-tension**	**MAP <70**	**Dopa ≤5 PE <100**	**Dopa > 5 Epi ≤ 0.1 NE ≤ 0.1 PE 100-300**	**Dopa>15 Epi>0.1 NE>0.1 PE>300 VP>0.01**	
Neuro **GCS**	**15**	**13-14**	**10-12**	**6-9**	**<6**	
Serum Creatinine **-OR-** **Urine Output**	**<1.2**	**1.2-1.9**	**2.0-3.4**	**3.5-4.9** **<500cc/ day**	**□5.0** **<200cc/ day**	
						Total

1. For patients on supplemental oxygen, add 0.03 to the room air FiO2 (0.21) for each liter of nasal cannula oxygen (e.g. 2 liters = FiO_2 of 0.27). Face mask FiO_2 is whatever amount is being delivered (e.g. 40% face mask = FiO_2 of 0.40). For non-rebreather face masks, use FiO_2 of 0.99.
2. Doses of dopamine (Dopa), epinephrine (Epi), norepinephrine (NE) are in micrograms/kg/min; phenylephrine (PE) is micrograms/min; vasopressin (VP) is U/min. Vasopressors must have been administered for at least one hour.

APPENDIX H: P/F from S/F Calculator

Table from Ellis Equation
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Ellis RK. JAP 1989

1/3	A	27300	35100	46800	49879	53300	57124	61425	66300	71871	78300	85800	94664	105300	118300	134550	155443	183300	222300	280800
0.333	B	27302	35102	46801	49880	53301	57125	61426	66301	71872	78301	85801	94664	105301	118301	134550	155443	183300	222300	280800

FiO2 \ SpO2	0.7	0.75	0.8	0.81	0.82	0.83	0.84	0.85	0.86	0.87	0.88	0.89	0.9	0.91	0.92	0.93	0.94	0.95	0.96
0.21	174	191	211	216	221	226	232	238	245	252	260	269	279	291	~~304~~	~~319~~	~~337~~	~~360~~	~~390~~
0.24	153	167	185	189	193	198	203	208	214	221	228	236	244	254	266	279	295	~~315~~	~~341~~
0.27	136	148	164	168	172	176	180	185	190	196	202	209	217	226	236	248	262	280	~~303~~
0.3	122	133	148	151	155	158	162	167	171	177	182	189	196	203	213	223	236	252	273
0.35	105	114	127	129	132	136	139	143	147	151	156	162	168	174	182	191	202	216	234
0.4	92	100	111	113	116	119	122	125	129	132	137	141	147	153	159	168	177	189	205
0.45	81	89	98	101	103	106	108	111	114	118	121	126	130	136	142	149	157	168	182
0.5	73	80	89	91	93	95	97	100	103	106	109	113	117	122	128	134	142	151	164
0.55	67	73	81	82	84	86	89	91	94	96	99	103	107	111	116	122	129	138	149
0.6	61	67	74	76	77	79	81	83	86	88	91	94	98	102	106	112	118	126	136
0.65	56	62	68	70	71	73	75	77	79	81	84	87	90	94	98	103	109	116	126
0.7	52	57	63	65	66	68	70	71	73	76	78	81	84	87	91	96	101	108	117
0.75	49	53	59	60	62	63	65	67	69	71	73	75	78	81	85	89	94	101	109
0.8	46	50	55	57	58	59	61	63	64	66	68	71	73	76	80	84	89	95	102
0.85	43	47	52	53	55	56	57	59	61	62	64	67	69	72	75	79	83	89	96
0.9	41	44	49	50	52	53	54	56	57	59	61	63	65	68	71	74	79	84	91
0.95	39	42	47	48	49	50	51	53	54	56	58	60	62	64	67	71	75	80	86
1	37	40	44	45	46	47	49	50	51	53	55	57	59	61	64	67	71	76	82

To calculate FiO2 for a non-intubated patient:

.21+(3.5 x per liter O2) = FiO2

Calculate PO2/FiO2 by finding SpO2 along the top row and calculated FiO2 along the left vertical axis and finding the intersection of the two.

SpO2/FiO2 ratio should only be analyzed if the SpO2 is < 97%. At values of 97% or greater there is no longer an interpretable relationship between SpO2/FiO2 and PaO2/FiO2

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