Administration of Intravenous Vitamin C in Novel Coronavirus Infection and Decreased Oxygenation

AVoCaDO

Protocol Number: 001

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STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and applicable United States (US) Code of Federal Regulations (CFR). The Principal Investigator(s) will assure that no deviation from, or changes to the protocol will take place without documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

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

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Administration of Intravenous Vitamin C in Novel Coronavirus Infection and Decreased Oxygenation (AVOCADO)
Study Description:	A novel coronavirus (SARS-CoV-2) has been recently identified to cause a new respiratory disease, Coronavirus disease-19 (COVID-19), and declared pandemic by the World Health Organization (WHO) on March 11, 2020. While the current mortality rates are unknown, a certain percentage of COVID-19 patients develop the Acute Respiratory Distress Syndrome (ARDS) leading to respiratory failure and death in high risk subjects such as elderly, and immunocompromised individuals. Previous research has shown that high dose intravenous vitamin C (HDIVC) may benefit in patients with acute lung injury (ALI) and ARDS. However, it is not known if early administration of HDIVC could prevent progression to ARDS. We hypothesize that intravenous administration of HDIVC is safe in COVID-19 subjects given early or late in the disease course and may reduce the risk of respiratory failure requiring mechanical ventilation and development of ARDS.
Objectives:	To assess the safety, tolerability, and efficacy of a 96-hour intravenous vitamin C infusion protocol (50 mg/kg every 6 hours) in patients with COVID-19 and evidence of respiratory dysfunction.
Endpoints:	**Primary Endpoint: Incidence of treatment related adverse events (TRAEs) during HDIVC infusion defined as**

- Acute kidney injury (increase in serum creatinine 3x baseline prior to initial HDIVC dose, hemolysis, iatrogenic hypoglycemia, pain at swelling site of infusion, crystalluria on urinalysis (UA) after last HDIVC dose
-

Secondary Endpoints:
Biochemical Hepatic Safety (assessed days 1-4):

- Change in liver enzymes (AST/ALT/ALP/TBILI)at baseline compared with value after last HDIVC dose

Hematological and Coagulation Safety Profile (assessed days 1-4)

- Change in White blood cell count, Hemoglobin, Hematocrit, Platelet count, INR, fibrinogen during HDIVC infusion

Tolerability of HDIVC defined as:

- Need for dose reduction, headache, dizziness, dry mouth, nausea, diarrhea, vomiting, flushing, rash, or hypotension during study infusion, assessed at hours 24, 48, and 96

Efficacy indicators of HDIVC, stratified to subjects that receive HDIVC with mild hypoxemia (SFR >250) versus severe hypoxemia (SFR <250

- Ventilator-free days at day 28
- ICU-free days at day 28
- Hospital-free days at day 28
- All-cause mortality at day 28
- Oxygenation
    - Change in SpO2/FiO2 (S/F) ratio at baseline compared with value after last dose of HDIVC
- Inflammation
    - Change in serum c-reactive protein (CRP), LDH, d-dimer, neutrophil/lymphocyte ratio, and ferritin at baseline compared with value after last dose of HDIVC

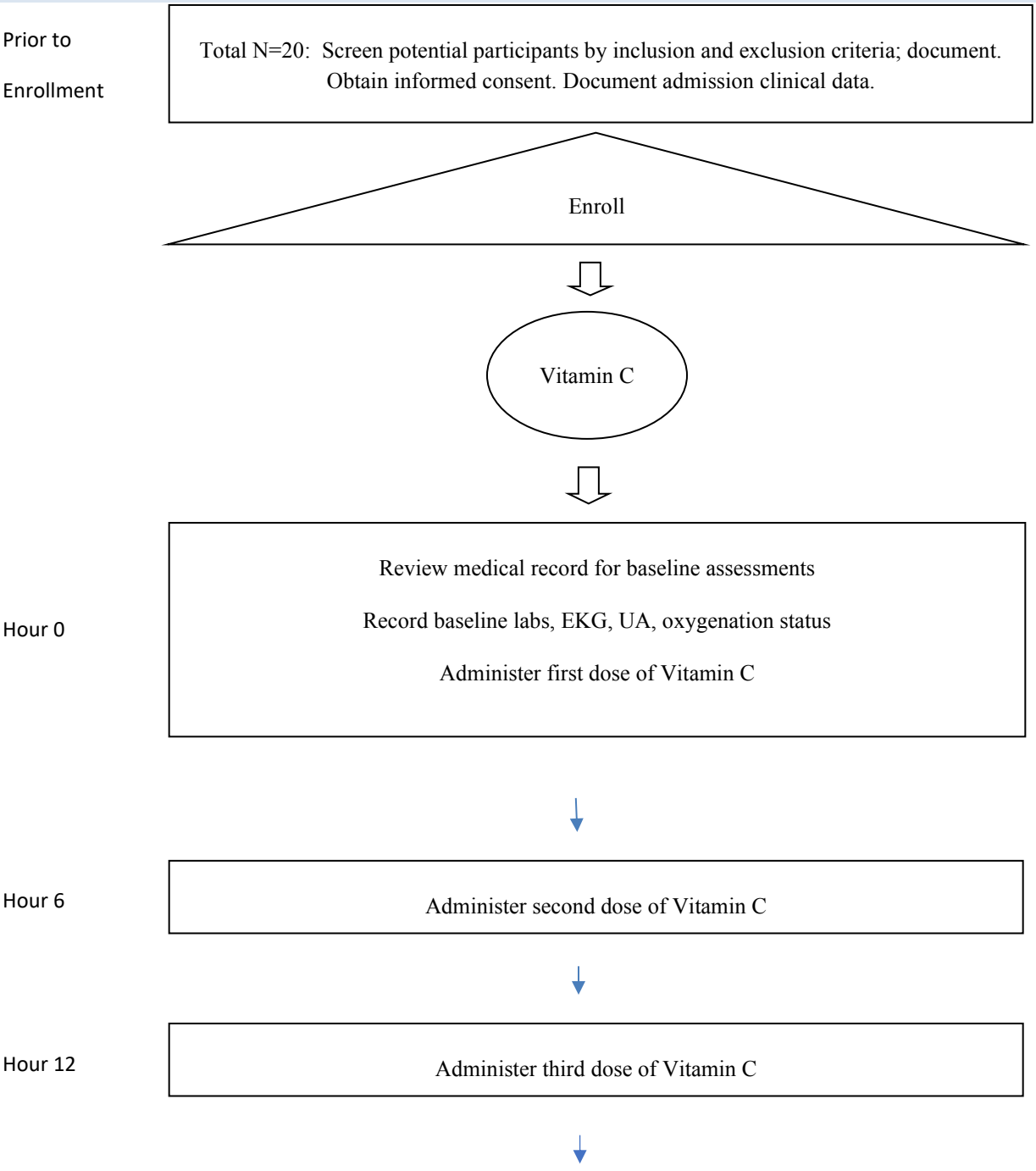
Study Population:	A total of up to 20 adult subjects with COVID-19 and documented evidence of decreased oxygenation will be enrolled in the study based on feasibility. All participants will be enrolled at the Central Virginia Veterans Administration Health System in Richmond, VA (RICVA).
Phase:	Phase 1
Description of Sites/Facilities Enrolling Participants:	Subjects will be recruited from the Emergency Department (ED), inpatient medicine unit, intensive care unit (ICU) admitted to the COVID-19 specific hospital floor or ICU. RICVA is a tertiary level medical center that serves more than 200,000 veterans annually in 52 cities and counties covering 22,515 miles in central and southern Virginia and parts of North Carolina.

Intervention: Participants will receive intravenous Vitamin C (50 mg/kg sterile L-ascorbic acid mixed in 5% dextrose in water) every 6 hours. Active treatment will continue for 96 hours, discharge from study hospital, study withdrawal, or death, whichever comes first.

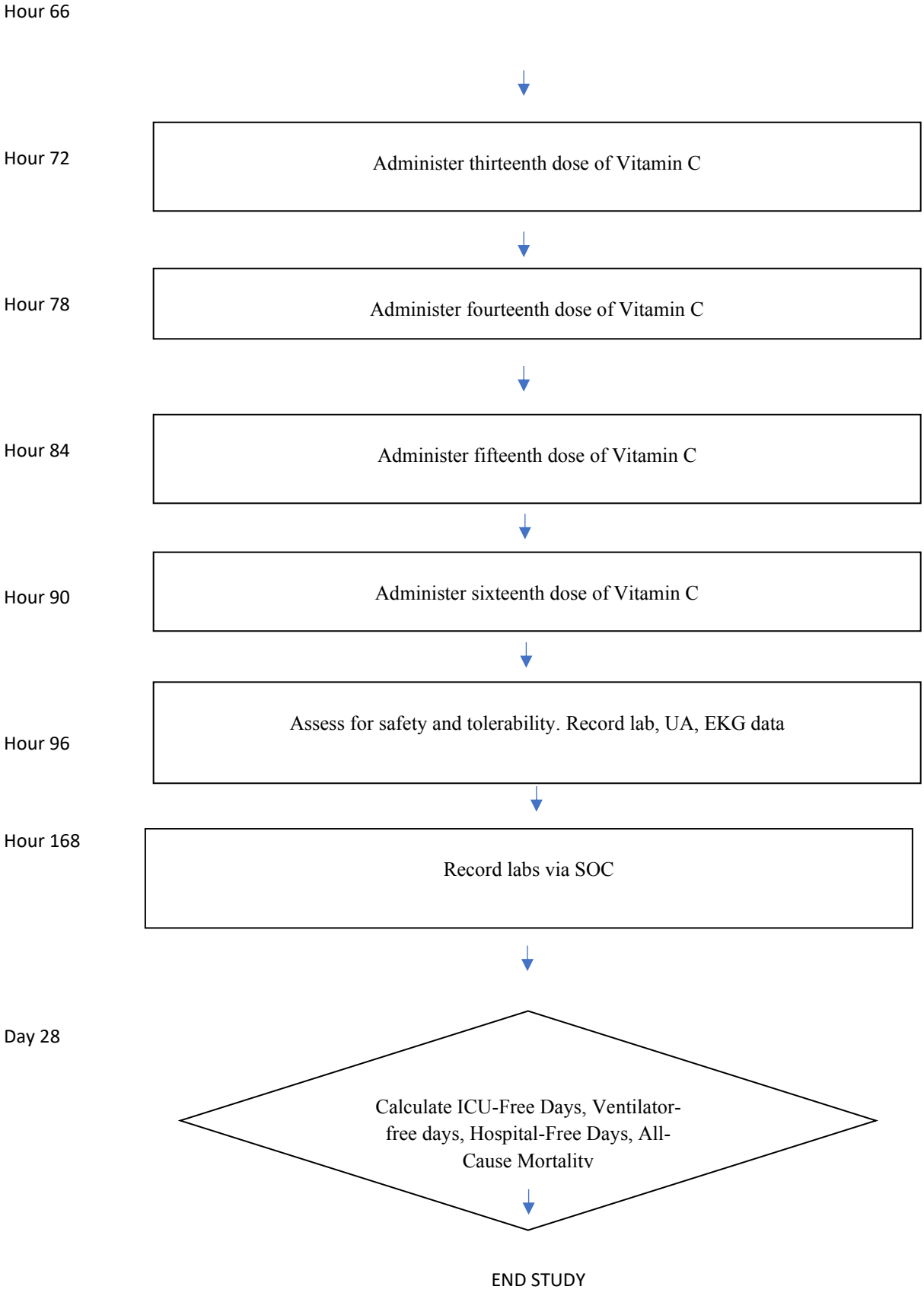
Study Duration: 28 days

Participant Duration: 28 days

1.2 SCHEMA




Hour 18
Administer fourth dose of Vitamin C
Hour 24
Administer fifth dose of Vitamin C. Assess for safety and tolerability
Hour 30
Administer sixth dose of Vitamin C
Hour 36
Administer seventh dose of Vitamin C
Hour 42
Administer eighth dose of Vitamin C
Hour 48
Assess for safety and tolerability. Record labs
Administer ninth dose of Vitamin C
Hour 54
Administer tenth dose of Vitamin C
Hour 60
Administer eleventh dose of Vitamin C
Administer twelfth dose of Vitamin C


Hour 66
Hour 72
Administer thirteenth dose of Vitamin C
Hour 78
Administer fourteenth dose of Vitamin C
Hour 84
Administer fifteenth dose of Vitamin C
Hour 90
Administer sixteenth dose of Vitamin C
Hour 96
Assess for safety and tolerability. Record lab, UA, EKG data
Hour 168
Record labs via SOC
Day 28
Calculate ICU-Free Days, Ventilator-free days, Hospital-Free Days, All-Cause Mortality
END STUDY

1.3 SCHEDULE OF ACTIVITIES (SOA)

Assessments	Hour 0	Q 24 hrs 1st 7 days or in ICU	Hour 48	Hour 96	Hour 168	Day 28
VS: BP, HR, MAP, RR, Temp., O2 sats, CVP, Glasgow Coma Scale, S/F ratio or P/F ratio if feasible	R/S	S	S	S		
Body Weight	S					
I/Os Total and Urine and urinalysis for crystalluria	R/S	S	S	R/S		
Assessment of Renal Function with serum creatinine, urine output and Use of Dialysis	R/S	S	S	R/S		
Labs: Na+, K+, BUN, Cr, WBC, Hgb, Hct, Platelets, PT/INR, PTT, CRP, ferritin, procalcitonin, LDH, d-dimer	R/S	S	S	R/S	R/S	
Bilirubin Total, AST, ALT, ALP, albumin	R/S	S	S	R/S		
All concomitant medications including: Corticosteroids, N-acetylcysteine, Antibiotics, Thiamine, Albumin, norepinephrine, vasopressin, other off-label or research COVID-19 medications	R	R	R	R		
AE/SAE/Tolerability Assessments	R	R	R	R		
ICU/Hospital Free Days						R
Glucose Monitoring	S	S	S	S	S	
EKG Monitoring	R/S	S	S	R/S		
All-Cause Mortality						R

S = standard of care (when feasible) R = research
R/S = if not ordered as routine care, labs will be ordered/obtained for research by treating provider or study personnel

2 INTRODUCTION

2.1 STUDY RATIONALE

The purpose of this study is to assess the safety, tolerability, efficacy of intravenously infused ascorbic acid (HDIVC) therapy for patients with COVID-19 and decreased oxygenation. COVID-19 is a rapidly evolving pandemic with numerous prediction models suggesting potential shortages in ventilators, ICU beds, and high rates of mortality if effective treatments are not developed quickly. A therapy is urgently needed to be given at disease onset in order to halt the infectious and inflammatory process, reduce risk of intubation, and development of ARDS. By administering the infusion at the first objective sign of worsening oxygenation, documented by change in S/F ratio or decreased pulse oximetry at baseline, HDIVC may reduce the inflammatory process and development of respiratory failure requiring intubation. We will also enroll patients already in respiratory failure on ventilators and document safety and tolerability, therapy demonstrating that HDIVC is safe in COVID-19 subjects early or late in the disease course. Due to the difficulty of running a study in a pandemic of highly infectious disease, we will use non-invasive measurements of deoxygenation, (i.e., Pulse Oximetry Saturation/Fraction of inspired oxygen, SFR) as opposed to obtaining an arterial blood gas to calculate P_aO_2/F_iO_2 ratio. The S/F ratio has been correlated with ARDS in previous studies. By measuring ventilator and ICU-free days, we can potentially signal efficacious endpoints that could be used in larger clinical trials needed to answer a crucial therapeutic question—can early administration of HDIVC in COVID-19 lead to faster recovery or improve outcomes? Moreover, we will document change in inflammatory markers that are elevated in COVID-19 (d-dimer, CRP, LDH, liver enzymes, and ferritin) to develop a mechanistic understanding and risk stratification of response to HDIVC infusion. Ultimately, if HDIVC is deemed safe in COVID-19 subjects, a larger clinical trial will be urgently needed, and AVOCADO will provide the framework to quickly up-scale in the context of an unprecedented pandemic and strain on health systems.

2.2 BACKGROUND

Brief Overview of COVID-19 Pandemic

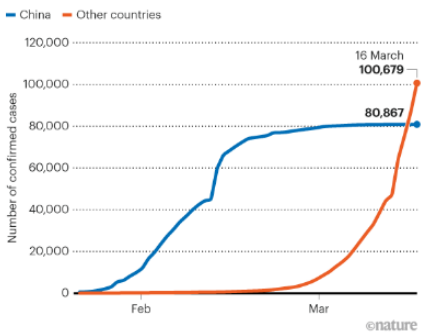


Figure 1. Rapid spread of SARS-CoV-2 as of 03/16/20.

In December 2019, a cluster of pneumonia cases were reported in Wuhan, China, and a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified.[1] The World Health Organization (WHO) designated the respiratory disease as coronavirus disease (COVID-19) on February 12, 2020.[2] Numerous reports have since characterized the COVID-19 clinical syndrome, ranging from asymptomatic/mild disease to severe disease leading to respiratory failure requiring mechanical ventilation, acute-respiratory distress syndrome (ARDS), multi-organ failure, sepsis, and death.[3–5] On March 12, 2020, WHO declared COVID-19 a pandemic as the virus had spread to 6 continents, with outbreaks in China, South Korea, Iran, Europe, and the United States (Figure 1).[6,7]

	No. (%) of patients[a]
Clinical measures	
Acute respiratory distress syndrome (ARDS)[b]	
None	1 (4.8)
Mild	2 (9.5)
Moderate	6 (28.6)
Severe	12 (57.1)
Ratio of arterial oxygen concentration to the fraction of inspired oxygen (range)	
At admission to ICU	169 (69-492)
At nadir	108 (58-247)
Use of noninvasive positive pressure ventilation	4 (19.0)
Use of high-flow oxygen therapy >15 L/min	1 (4.8)
Required mechanical ventilation	15 (71.0)
Among patients requiring intubation for mechanical ventilation	
Hospital days prior to intubation, mean (range), d	1.5 (0-12)
Use of prone positioning for ARDS	8 (50.0)
Use of inhaled epoprostenol for ARDS	5 (31.3)
Use of vasopressors	14 (67.0)
Absolute lymphocyte count at nadir (range), /μL	525 (180-1100)
Evidence of co-infection[c]	
Bacterial	1 (4.8)
Viral	3 (14.3)
Acute kidney failure[d]	4 (19.1)
Cardiomyopathy[e]	7 (33.3)
Acute hepatic injury[f]	3 (14.3)
Seizures	1 (4.8)
Length of follow-up, mean (range), d	5.2 (1-10)
Outcomes	
Died	11 (52.4)
Survived to transfer out of ICU	2 (9.5)
Remains critically ill and requires mechanical ventilation	8 (38.1)
Length of follow-up for those who survived or remain critically ill, mean (range), d	7.5 (5-10)

Figure 2. Clinical Measures in Critically Ill COVID-19 Patients in Washington State.[8]

While only a small percentage of COVID-19 patients develop severe respiratory failure, the predicted surge in patient volume is causing concern about access to intensive-care unit (ICUs) and mechanical ventilators. Italy has already reached maximum capacity of ICU beds and early reports suggest rationing of ventilators in certain hospitals is occurring.[9–11] Guidelines have been developed to help physicians manage this crisis situation.[12] Recent modeling based on data from China suggests the United States lacks the capacity to manage all COVID-19 patients due to lack of ICU beds and ventilators.[13] Initial experiences with critically ill COVID-19 patients in the United States has recently been described, with most deaths due to ARDS (Figure 2). A safe, effective, and inexpensive therapy is urgently needed that can alter the natural history of the disease process and reduce strain on health systems.

Preliminary Data To Support the Use of HDIVC in ARDS

HDIVC prevents pulmonary neutrophil infiltration in a feces-induced peritonitis (FIP) model of sepsis/ARDS (Figure 3).

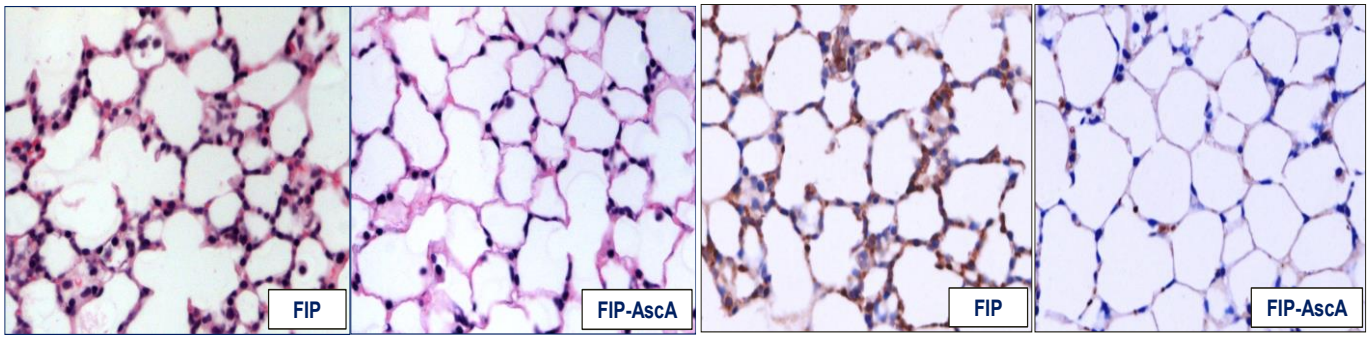


Figure 3a: AscA (200 mg/kg) infused 30 min following onset of feces Induced peritonitis (FIP) attenuates sepsis-mediated acute lung injury in wild type mice at 16 hours. (H&E stain, 40X magnification)

Figure 3b: AscA (200 mg/kg) infused 30 min after feces induced peritonitis significantly attenuated neutrophil sequestration in muring lung at 16 hours. (Anti-murine PMN monoclonal antibody, 40X Mag)

HDIVC has been proposed to have a pleotropic effect in ARDS[14] (Table), including acting as a stress hormone (required for synthesis of norepinephrine, dopamine, vasopressin)[15], improves endothelial function by decreasing circulating thrombomodulin[16], promotes lymphocyte maturation,[17] and reduces cytokines driving inflammatory pathways in ARDS,[18] including IL-6 in patients with pneumonia.[19]IL-6 is emerging as a key cytokine in COVID-19 pneumonia and ARDS and has been associated with increased morbidity and mortality.[20]

Table: Potential Mechanisms of Vitamin C in ARDS (adopted from Kashiouris et al)

Physiology/Mechanism	Description	Potential Mechanism in Covid-19

Antioxidant	Reverses oxidation of lipids by the neutrophil reactive oxygen species (ROS). May prevent oxidation of lipids, proteins, and DNA	Attenuates ROS driving ARDS in Covid-19
Stress Hormone Synthesis	Essential co-factor in synthesis of norepinephrine from dopamine, increases adrenergic receptor activity, cofactor in hydroxylation of L-tyrosine to L-DOPA, cofactor in vasopressin synthesis, high concentration in brain and adrenal glands	New reports suggest Covid-19 patients in ICU have high vasopressor requirements (70%), HDIVC may lead to reduction in time on vasopressor due to increased endogenous synthesis
Regulation of cellular gene expression in response to hypoxia and stress	Needed to downregulate hypoxia induced factor 1alpha (HIF1a), which is a protein-transcription factor that regulates hundreds of genes in response to hypoxia and stress and is increased in sepsis, shock, and ARDS	In intro study of SARS-CoV-1, the spike protein (also present in SARS-CoV-2) greatly enhanced expression of HIF1a
Connective tissue maintenance	Vital in wound healing and catalyzing formation of procollagen and elastin, stimulates production of new collagen, induces fibroblast collagen gene expression	SARS-CoV-2 destruction of barrier function in lung tissue is key event leading to pneumonia/ARDS
Carnitine biosynthesis	Co-factor in synthesis of L-carnitine, which transports lipids into mitochondria and can down-regulate tumor necrosis factor-alpha (TNFa), a key driver of inflammation	TNFa levels elevated in hospitalized Covid-19 subjects, and downregulation may reduce inflammatory process
Phagocytic cell function	Severe vitamin C deficiency (Scurvy) associated in impaired ability of neutrophil to phagocytose and generate reactive oxygen species, thereby decreasing microbiota destruction	The spike protein may trigger dysfunction of immune cells based on *in vitro* data in SARS-CoV-1
Immune cell clearance	Promotes neutrophil apoptosis instead of necrosis via activation of caspase-3 proteins; decreases circulating cell-free DNA, which can cause end-organ damage	Inflammatory cells with necrosis described in early autopsy reports
Lymphocytic function	May promote lymphocytic proliferation, differentiation, and maturation. Decreased lymphocyte count is emerging as hallmark sign of COVID-19	Increasing lymphocyte count may help with viral clearance
Endothelial function	HDIVC decreases plasma syndecan-1 levels and glycocalyx shedding, which is a key mechanism in development of ARDS and fluid entering the alveoli space	Covid-19 autopsy data shows severe damage to lung tissue with disruption of endothelial barrier similar in other ARDS etiologies

HDIVC Increased Clearance of Bacteria from Circulation in a Preclinical Model of Sepsis/ARDS

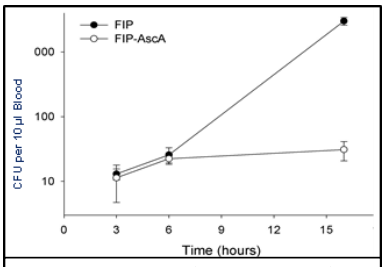


Figure 4: Blood cultures from septic mice after FIP onset. AscA treated mice (200 mg/kg) show significantly lower colony forming units per 10 µl of blood compared to untreated septic animals.

In the same murine model of sepsis, blood was obtained by cardiac puncture at the time of euthanasia using sterile technique varying time points after induction of feces induced peritonitis with or without associated HDIVC administration (Figure 4). 10 µl of blood was plated on agar plates and colonies counted after overnight incubation. HDIVC treated mice had a highly significant decrease in colony counts compared to untreated mice ($p< 0.01$). Bronchoalveolar lavage in these mice also demonstrated an increased in alveolar protein content after induction of FIP and protection from HDIVC (data not shown).

Human Data to Support the Safety and Efficacy of HDIVC in Humans with Sepsis/ARDS

Figure 5

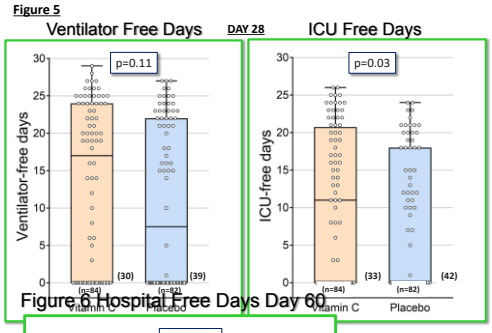
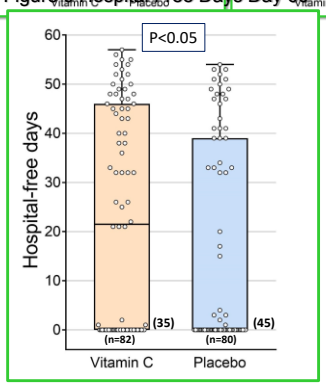


Figure 6 Hospital Free Days Day 60



Fowler et al. JAMA. 2019 PMID: 31573637

In the CITRIS-ALI study[21], a multi-center, randomized, double blind placebo controlled trial, ARDS patients resulting from sepsis who received HDIVC (at 50 mg/kg every 6 hours for 4 days, n=84) had higher ventilator-free days at day 28 than placebo though not significant (Figure 5). However, CITRIS-ALI showed that patients treated with HDIVC had significantly more ICU free days at day 28 than placebo. (Figure 5) and hospital free days to day 60 (Figure 6) found that patients receiving vitamin C had a higher number of ICU free days at day 28 and a higher show a statistically significant reduction in mortality and ICU-free days at 28 days in ARDS subjects receiving HDIVC compared to placebo. Further trials are currently in the planning and enrolling phase for HDIVC in sepsis and ARDS. A recent trial using the trio of lower dosage vitamin C plus thiamine and hydrocortisone (VITAMINS) did not show improvement in time alive or vasopressor free days in a multicenter, randomized, open-label trial. However, this trial gave a lower dose of HDIVC than CITRIS-ALI, was open-label, and not in an ARDS-specific cohort.[22] Furthermore, patients treated with HDIVC showed a significantly decreased organ failure as assessed by the sequential organ failure assessment score (SOFA) (Figure 7). Most importantly, the CITRIS-ALI trial found that patients treated with HDIVC had a significantly improved survival at days 28 (Figure 8). Thus with the clinical data in patients with acute lung injury (ARDS) we have justification to proceed with the application of HDIVC in COVID-19 positive patients.

Figure 7

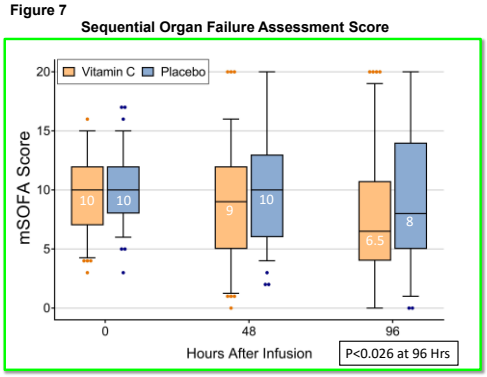
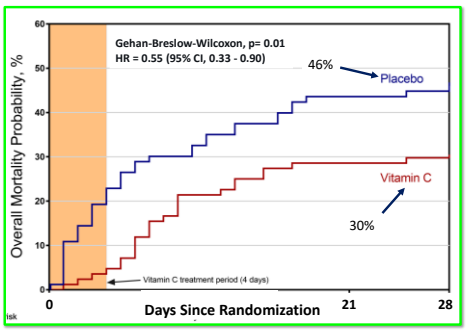


Figure 8 CITRIS-ALI 28-Day Mortality



Fowler et al. JAMA. PMID: 31573637

Data for Use of High Dose Intravenous Vitamin C in Viral Respiratory Disease

High dose vitamin C has also been given in a case of viral-induced ARDS in a patient who developed severe respiratory failure that required mechanical ventilation and extracorporeal membrane oxygenation (ECMO). Rapid improvement in oxygenation was noted early after HDIVC, which was given for 7 days total. The patient had no side effects from HDIVC or any long-term sequalae.[23]

Vitamin C supplementation has been given in numerous trials for viral respiratory disease with mixed results. In one study with marine recruits (n=674), a randomized, double-blind study of oral vitamin C at 2.0g/day reduced the reported pneumonia cases compared with placebo.[24]A randomized clinical trial is under way in Wuhan, China to administer 12g of intravenous vitamin C every 12 hours for 7 days compared with placebo (NCT04264533).

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Potential Physical Risks of Ascorbic Acid Infusion: dry mouth, nausea, vomiting, dizziness, headache. pseudohyperglycemia (if checked with point of care glucose testing), crystalluria.

Potential Psychological, Social, Legal Risks of Ascorbic Acid Infusion: No psychological, social or legal risks are identifiable from an extensive literature search.

Risks of Blood Draws: Not applicable to study as blood draws will be standard of care based on treating physician.

Glucose Monitoring Plan:

Guidance for blood glucose monitoring in patients enrolled in the AVOCADO 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.

Inpatient units not using the StatStrip POC glucometer should follow these guidelines:
Guidance for blood glucose monitoring in patients enrolled this study:

- Critical Care and Inpatient Unit Nursing and Treating Physician must be informed of vitamin C's effect on point of care (glucometer) blood glucose and arterial blood gas glucose point of care values. The PI(s) will instruct the ordering providers about how to monitor blood glucose and insulin management in the study.

- 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 as a part of standard of care will have metabolic glucose screening on the schedule determined by the attending/ordering physician
- 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.
- Point of care glucose testing may resume 36 hours after the last infusion of study drug.

2.3.2 KNOWN POTENTIAL BENEFITS

Most observational studies suggest a mortality benefit in inpatient subjects given Vitamin C for serious infections and burns. 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.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

The identifiable risks arising from exposure to intravenous ascorbic acid infusion are low. 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).

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Vitamin C infusion will be safe and tolerable in COVID-19 subjects	Documented side effects and serious adverse events at hours 24, 48, 96 (based on Ascor® package insert)	HDIVC infusion may cause dry mouth, nausea, vomiting, dizziness, headache, flushing, rash, diarrhea. HDIVC can be excreted from kidney and lead to crystalluria, so will monitor urine pH via urinalysis.
Secondary		
Efficacy of HDIVC in COVID-19	Ventilator-free days ICU-free days Mortality at 28 days	Previous research has suggested that HDIVC may reduce time on ventilator, therapy reducing ICU and mortality, which is critical in

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
		setting of a pandemic caused by respiratory disease
Secondary		
Efficacy of HDIVC in COVID-19	Reduction in Inflammation Change in baseline CRP and ferritin compared with hour 168 (day 7)	Previous research has suggested that elevated CRP and ferritin at baseline are associated with increased risk of ARDS and mortality in COVID-19
Secondary		
Efficacy of HDIVC in COVID-19	Improvement in oxygenation Change in S/F ratio at baseline compared with hour 96	Stabilization or improvement in the S/F ratio may be an indicator of early recovery and that subjects will not require mechanical ventilation

4 STUDY DESIGN

4.1 OVERALL DESIGN

Up to 20 subjects with COVID-19 and decreased oxygenation will receive *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 and occur every 6 hours for a total of 16 doses. Standard of care therapy will be followed and recorded when available.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

The purpose of this study is to assess the safety, tolerability, efficacy of intravenously infused ascorbic acid therapy for patients with COVID19 and decreased oxygenation. By administering the infusion at the first objective sign of worsening oxygenation, documented by S/F ratio, decreased pulse oximetry at baseline, HDIVC may reduce the inflammatory process and development of respiratory failure requiring intubation, which is critical in a pandemic when ventilators, ICU beds, and medical staff may be in short supply. We will also enroll patients already in respiratory failure on ventilators and document ventilator-free days, demonstrating proof of concept that COVID19 patients with ARDS may have similar recovery compared to sepsis-induced ARDS in other HDIVC clinical trials.

4.3 JUSTIFICATION FOR DOSE

Dosing and bio-distribution data in humans show that pharmacological concentrations of vitamin C can only be attained following intravenous administration. 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.[25] 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.

4.4 END OF STUDY DEFINITION

Active treatment will continue for 96 hours, discharge from study hospital, study withdrawal, or death, whichever comes first. All subjects will be followed to day 28 for collection of outcomes data even though the study intervention will be completed by 96 hours from enrollment.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

- Hospitalized with diagnosis of COVID-19 based on positive real-time polymerase chain reaction (RT-PCR) of nasal, oropharyngeal, or BAL specimen
- Decrease in oxygenation:
    - Mild deoxygenation defined as S/F ratio decreased by 25% from baseline on admission, or SaO2 <95% breathing ambient air on admission (SFR ≥250 prior to study drug infusion)
    - Severe deoxygenation defined as respiratory failure requiring mechanical ventilation
        - SFR <250 prior to study drug infusion
    - Non-childbearing potential or childbearing potential with a negative pregnancy test at screening, and using a reliable method of contraception (i.e., abstinence, hormonal contraception, IUD, or vasectomized partner)

5.2 EXCLUSION CRITERIA

1. Known allergy to Vitamin C
2. Inability to obtain consent from patient or next of kin
3. Chronic kidney disease, stage IV or above (eGFR <30)
4. Presence of diabetic ketoacidosis, use of insulin infusion, or frequent need for point-of-care glucose monitoring (>6 times/24-hour period) as determined by treating physician
5. History of glucose-6-phosphate dehydrogenase (G6PD) deficiency
6. Admission ALT or AST greater than 10 times the upper limit of normal
7. Active or history of kidney stone (nephrolithiasis or crystalluria) within the past 12 months
8. Pregnancy
9. Enrolled in another COVID-19 clinical trial that does not allow concomitant study drugs

5.3 LIFESTYLE CONSIDERATIONS

Not applicable to this study.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently enrolled in the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the

Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE). Subjects will be documented in the Study Screening Log when all Inclusion Criteria are met.

Individuals who do not meet the criteria for participation in this trial (screen failure) because of exclusion criteria such as failure to obtain consent, inability to locate the legally authorized representative (LAR), or delay in diagnosis may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Patients with COVID-19 will be recruited from the Emergency Department (ED), the medicine inpatient, and the intensive care units (ICU) at Central Virginia VA Health System in Richmond, Virginia (RICVA). RICVA is a large tertiary care medical center with a large referral base. Study personnel will review patients within the electronic medical record 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 ED, inpatient unit, or 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.).

For this study, we will not exclude subjects enrolled in other COVID-19 clinical trials as obtaining safety data of interactions with other medications has clinical merit. We will track all COVID-19 related medications given during this study, including a category for “other”. Subjects may be excluded from this study if other COVID-19 trials do not allow concomitant therapies or specifically exclude other COVID-19 treatments in protocol with or without washout phase. The IRB will be informed of initial enrollment and study investigators will follow IRB recommendations when multiple experimental studies are available to subjects.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

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) could be inserted depending on judgment of attending physician.

6.1.2 DOSING AND ADMINISTRATION

1) **First study drug dose** (L-ascorbic acid) will be considered "Dose 1" and will be administered within 6 hours of enrollment 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 medicine inpatient unit or 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 (+/- 6 hours).
   a) Timing of Dose 2 will be triggered by the provider order for every 6-hour administration and will therefore be listed on the bedside MAR. As such, timing of Dose 2 may be out of the +/- 6 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.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

VA Investigational Drug and/or VA Inpatient Pharmacy will coordinate acquisition of sterile L-ascorbic acid for infusion from the manufacturer.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

ASCOR vials contain 25,000 mg/50mL of ascorbic acid and is supplied as pharmacy bulk packaging (PBP). The diluted ASCOR solution should appear colorless to pale yellow.

6.2.3 PRODUCT STORAGE AND STABILITY

ASCOR is light sensitive so light exposure should be minimized and stored per package insert instructions:

Store under refrigeration (2°C to 8°C); protect from light with amber IV bag cover. Infusion solutions prepared by the IDS Pharmacy in the Pharmacy IV admixture suite will expire in 24 hours if refrigerated and light-protected.

There is published data establishing the stability of admixtures of ascorbic acid to be at least 24 hours stored at room temperature or refrigerated. Also, Dr. Alpha Fowler has unpublished data that was obtained prior to an earlier IND trial (IND #113856) which demonstrates the stability of samples prepared in the same manner as described below.

6.2.4 PREPARATION

Investigational Pharmacy will mix L-ascorbic acid once every 24 hours in four 50 ml hooded infusion bags and stored in the inpatient unit or ICU in the dark at 4ºC. The prepared IV bags will have the IV tubing attached and primed b*y the bedside nursing staff*. At designated infusion time, Nursing will infuse the contents of the light-shielded agent via infusion pump through tubing over 30 minutes. Prior studies performed have shown that L-ascorbic acid prepared for infusion in this way remains stable with no quantifiable oxidation.

Study drug will be prepared in 50 ml D5W bags, USP, using aseptic technique according to USP 797requirements. All ASCOR pharmacy bulk package vials utilized will be discarded within 4 hours or sooner as per the ASCOR package insert.

The volume of ascorbic acid to be added to the IV bag will be calculated. This same amount of fluid will be drawn out of the D5W 50 mL bag and discarded. Then, the calculated volume of ascorbic acid will be added to the IV bag. As much air as possible will be removed from the IV bag using an empty syringe with needle attached (in order to prevent oxidation). The IV bag will be covered with a light-protective shroud.

Microbiologic Controls:

Investigational Pharmacy has an ISO Class 5 laminar airflow hood (Nuaire Class II Type A2 biological safety cabinet) in a segregated compounding area. This biological safety cabinet is recertified every 6 months. Compounding personnel must successfully complete media fill testing and gloved, finger-tip sampling as per USP 797 regulations.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Not applicable to this study. An amendment to the study protocol with randomization may be added if the safety endpoint is met and secondary endpoints suggest a clinical benefit.

6.4 STUDY INTERVENTION COMPLIANCE

Adherence to the protocol will be assessed and verified using participant drug log, review of electronic medical records and review of the eCRF.

6.5 CONCOMITANT THERAPY

Any concomitant medications as part of standard of care (particularly antibiotics, oxygen therapy, off-label COVID-19 medications such as hydroxychloroquine, chloroquine, azithromycin, remdesivir, tocilizumab, lopinavir/ritonavir, mavrilimumab, convalescent serum, glucose infusion, insulin, albumin, corticosteroids, N-acetylcysteine, norepinephrine, vasopressin) provided will be recorded.

6.5.1 RESCUE MEDICINE

Not applicable.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

If subjects do not tolerate the drug due to side effects such as nausea, vomiting, flushing, rash, headache, diarrhea, develops crystalluria on urinalysis, or QTc prolongation >50% baseline, we will reduce the subsequent doses by 50%. If the QTc is greater than 600 msec at any time, the drug will be discontinued.

Loss of indwelling venous/arterial catheter or peripheral intravenous access will also 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.

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 and is without a central line.

The study drug will be discontinued if a patient develops a metabolic acidosis unexplained by other etiologies (ie lactic acidosis secondary to septic shock). Determination of the presence of metabolic acidosis will be made by the clinical care team. Study drug will also be discontinued if primary care team or surrogate decision maker request withdrawal. The study drug will be discontinued if an allergic reaction during study infusion is documented, oxalate nephropathy is diagnosed (defined as rise in serum creatinine by 3 times baseline with oxalate crystals on urinalysis), an insulin drip is started, or hemolytic anemia develops (defined as drop in baseline serum hemoglobin >2 g/dL with laboratory evidence of hemolysis). Data collection will continue for these patients following withdrawal of study drug.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

7.3 LOST TO FOLLOW-UP

Not applicable to this study.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Screening
Subjects will be evaluated for entry into the study according to stated inclusion and exclusion criteria. Individuals who are identified during screening as not eligible for the study need not complete all screening procedures. The reason for ineligible status will be documented on the Screen Failure Log.

The following information will be obtained to evaluate each subject's qualifications for participation in the study:

- Demographic information including gender, date of birth, race, ethnicity
- Medical and medication history over the past 30 days, including clinical data on admission
- Collection of blood for chemistry, hematology, coagulation, microbiology, in female subjects of childbearing potential, a pregnancy test (urine pregnancy test or blood human chorionic gonadotropin (hCG)
- Physical examination including height and weight (body mass index will be calculated based on these variables), and vital signs (systolic and diastolic blood pressure, pulse rate, respiratory rate, pulse oximetry, body temperature), respiratory exam (crackles yes/no, wheezing yes/no, increased work of breathing yes/no), abdominal exam (abdominal tenderness yes/no) neurological exam (Glasgow Coma Scale)
- Chest imaging findings (CXR with bilateral infiltrate yes/no ground glass opacities yes/no; CT chest with bilateral ground glass opacities yes/no) based on radiological attending report
- Mechanical ventilation (yes/no)
- Use of supplemental oxygen (yes/no)
- Review inclusion and exclusion criteria

- Signed, written informed consent (may be done by telephone or telehealth/virtual if limited access to patient or surrogate due to hospital visitation policies)

Assessments at Baseline (Hour 0):
- Documentation of concomitant medications (if applicable); including oxygen therapy (to calculate S/F ratio), antibiotics, corticosteroids, albumin, thiamine, norepinephrine, vasopressin, and N-acetylcysteine use, off-label or other study drugs for Covid-19 treatment
- Documentation of blood for hematology, coagulation, clinical chemistries
    - COVID-19 biomarkers include Ferritin, CRP, LDH, lymphocyte count, d-dimer (primary provider or study personnel to order if not available)
- Documentation of vital signs
    - Calculation of S/F ratio
- Documentation of mechanical ventilation status (yes/no)
- Documentation of EKG (study personnel to order if not available)
- Documentation of negative urine or serum pregnancy testing for human chorionic gonadotropin (hCG) if female and of childbearing potential

Treatment Day 1 (Hour 24):
- Documentation of concomitant medications (if applicable)
- Documentation monitoring of vital signs: blood pressure, temperature, heart rate, oxygen saturation
    - Calculate S/F ratio
- Documentation of mechanical ventilation status (yes/no)
- Documentation of blood test results for standard of care (SOC) hematology, coagulation, clinical chemistry
- Document urinalysis
- AE and tolerability review (phone interview may be conducted if feasible to reduce exposure of study personnel)

Treatment Day 2 (Hour 48):
- Documentation of concomitant medications (if applicable)
- Documentation of vital signs: blood pressure, temperature, heart rate, oxygen saturation
    - Calculate S/F ratio
- Documentation of mechanical ventilation status (yes/no)
- Documentation of blood for hematology, coagulation, clinical chemistry via SOC
- Document urinalysis
- AE and tolerability review (phone interview may be conducted if feasible to reduce exposure of study personnel)

Treatment Day 3 (Hour 72):
- Documentation of concomitant medications (if applicable)
- Documentation of vital signs: blood pressure, temperature, heart rate, oxygen saturation
    - Calculate S/F ratio
- Documentation of mechanical ventilation status (yes/no)
- Documentation of blood for hematology, coagulation, clinical chemistry via SOC
- Document urinalysis

- AE and tolerability review (phone interview may be conducted if feasible to reduce exposure of study personnel)

Treatment Day 4 (Hour 96, end of study infusion):
- Documentation of concomitant medications (if applicable)
- Documentation of vital signs: blood pressure, temperature, heart rate, oxygen saturation
    - Calculate S/F ratio
- Documentation of mechanical ventilation status (yes/no)
- Documentation of blood for hematology, coagulation, clinical chemistry via SOC (study team to order if not available)
- Document urinalysis (primary provider or study personnel to order if not available)
- Document EKG (primary provider or study personnel to order if not available)
- AE and tolerability review (phone interview may be conducted if feasible to reduce exposure of study personnel)

Study Day 7 (Hour 168):
- Documentation of mechanical ventilation status (yes/no)
- Documentation of blood for SOC hematology, coagulation, clinical chemistry (if available)
    - COVID-19 markers include CRP, ferritin, LDH, lymphocyte count, d-dimer (order by study personnel if not already done)

Study Day 28
- Phone call or chart review to document ventilator-free days, ICU-free days, hospital-free days, mortality

8.2 SAFETY AND OTHER ASSESSMENTS

Assessment of hepatic safety: We will follow the current guidance from FDA for assessment of liver safety in those with pre-existing hepatic impairment. Evaluation of Drug Induced Severe Hepatitis (eDISH): eDISH plots will be overlayed from drug treated arms at the end of the study to look for DILI signals. Those cases with bilirubin > 3x ULN and ALT > 8x ULN will be studied individually and adjudicated for DILI. These data will be provided for concordance to IRB.

For individual cases, the following rules will be followed for drug discontinuation for DILI:

If AST or ALT rise by > 50% from baseline but are less than 8 times the upper limit of normal and this is associated with less than 3 mg/dl rise in bilirubin, a hepatic panel will be repeated within 24 hours. If the AST and ALT continue to rise >100 IU/L over 24 hours or bilirubin rises by >3 mg/dl, the study drug will be discontinued.

If AST or ALT exceed 8 times the upper limit of normal at any time or bilirubin rises by 3 or more mg/dl from baseline within a 48-hour time frame, the study drug will be discontinued.

Assessment of infusion safety: If the subject has clinically significant side effects based on the L-ascorbic acid package insert, the infusion will be stopped, the primary provider and PI will be notified.

An allergic reaction with symptoms such as hypotension, rash, flushing, hives, dyspnea, swelling of mouth or lips will lead to stopping of the study drug, notification of PI and primary provider, documentation of SAE, and the medical record will be amended with new allergy

The most common adverse reactions are pain and swelling at the site of infusion. Nausea, vomiting, diarrhea, facial flushing, rash, headache, fatigue, or disturbed sleep can rarely occur. If clinically significant symptoms occur during the infusion, the infusion will be stopped, and subsequent doses reduced by 50% (100 mg/kg/day for 16 total doses). If symptoms continue at reduced dose, then the study drug will be discontinued.

If development of severe acute kidney injury (increase in serum creatinine 3.0 times baseline) with urinalysis showing evidence of oxalate nephropathy (new onset proteinuria, hematuria, or oxalate crystals), or kidney biopsy with oxalate crystal deposition in tubules or interstitium, then the infusion will be discontinued, and treatment started for oxalate nephropathy. A recently published systemic review found 5 cases of oxalate nephropathy in 2,360 HDIVC subjects, and all occurred at 3 times higher doses (60 grams/day or higher)[26] than described in protocol section 6.1.2.

If hemolysis is suspected based on reduction in hemoglobin by >2 grams from baseline with laboratory evidence of hemolysis (increased reticulocyte count, elevated LDH, indirect bilirubin, reduced haptoglobin), the study drug will be discontinued, and treatment initiated as needed.

If the patient is started on an insulin drip or it is deemed medically necessary to monitor blood glucose more than every 6 hours, then study infusion will be discontinued.

These events will be documented in the SAE form, AE form and CRF form after grading by the PI for severity as described in section 8.3.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

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

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of the investigator(s), it results in any of the following outcomes: death, a life-threatening adverse event, prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, or be life-threatening, may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

The following subsections will include a discussion of how AEs will be classified.

8.3.3.1 SEVERITY OF EVENT

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious"

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

8.3.3.3 EXPECTEDNESS

The Principal and Sub-Investigators will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

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

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

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

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The study personnel will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

Investigators will report all unanticipated problems that involve risk or harm to a research participant to the IRB in the required reporting time frame. The IRB will be notified within 5 business days of receiving notice of the unanticipated problem.
Investigators 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 investigator 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.

Examples of untoward clinical occurrences, or disease-related events (DREs) that are expected in the course of COVID-19 include: 1) transient or worsening hypoxemia, 2) agitation, 3) delirium, 4) diarrhea, 5) nosocomial infections, 6) skin breakdown, 7) acute hepatitis, 8) acute kidney injury that may require renal replacement therapy, 9) worsening of cardiopulmonary function that may require artificial cardiopulmonary support, 10) worsening hypotension that may require vasopressor support, and 11) acute thrombosis or coagulopathy. 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 sepsis and ARDS. 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., Sp02 ~85%), related to positioning or suctioning. This latter event would not be considered unexpected by nature, severity or frequency. These events will be captured in the eCRF.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

The study clinician will immediately report to the IRB any serious adverse event and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the IRB.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria from the time of consent to through study hour 168 until resolved, withdrawn from the study, death, or lost to follow up:

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

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing IRB. The UP report will include the following information:

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

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB within 48 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB within 5 business days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 5 business days of the IRB's receipt of the report of the problem from the investigator.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES FOR PRIMARY ENDPOINT

Not applicable to this study.

9.2 SAMPLE SIZE DETERMINATION

We will aim to enroll 20 patients to establish safety data and consider an amendment to the protocol for additional enrollment based on COVID-19 cases and further evaluation of secondary endpoints.

9.3 POPULATIONS FOR ANALYSES

We will stratify subjects for secondary analysis into subjects with mild decreased oxygenation, defined as SaO2 <95% on room air upon admission or requiring supplemental oxygenation and SFR ≥ 250. subjects with severe decreased oxygenation will be defined as SFR <250.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Continuous variables will be compared across groups using Student's T-test, ANOVA, or a Kruskal Wallis ANOVA depending on their distribution. Changes from baseline to time of measurement will be compared by analysis of covariance. Categorical variables will be measured by Fisher's Exact test. Correlations will be assessed by Spearman's coefficient. The Shapiro-Wilk test will be applied to determine normality. Parametric dependent means will be compared with paired Student's T-test and non-parametric means will be analyzed using Wilcoxon signed-rank test. 95% confidence intervals will be calculated and a p value of <0.05 will be considered statistically significant. SPSS and Stata will be used to conduct analysis.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The primary endpoint will be treatment related adverse events at end of study drug infusion, as defined in 9.4.3. Subjects will be stratified into two cohorts: mild deoxygenation and severe deoxygenation. Descriptive statistics will be applied.

9.4.3 SAFETY ANALYSES

Adverse Events

Adverse events will be classified into standard terminology using the Medical Dictionary for Regulatory Activities (MedDRA). Adverse events will be listed by reported verbatim term and MedDRA-preferred term, start and stop date and time, study day, duration, severity, relationship to study drug, and seriousness. Additionally, data may be grouped for analysis by different levels of the MedDRA hierarchy. The incidence of AEs, the incidence of treatment- related AEs, and the severity of AEs will be tabulated by cohort (mild versus severe deoxygenation). The number and percentage of patients with SAEs and treatment-related SAEs and patients who withdraw due to an AE will be tabulated.

Clinical Laboratory Testing

Clinical laboratory test parameters will be listed for individual patients. Baseline for clinical laboratory parameters will be defined as the last evaluation before the start of the initial dosing with study drug. Summary statistics, including mean absolute change from baseline, will be calculated for each parameter and summarized.

Vital Signs

Vital signs results (systolic and diastolic blood pressure, pulse rate, pulse oximetry, and body temperature) will be listed for individual patients. Each vital sign measure will be tabulated by evaluation time point. Baseline for vital signs measurements will be defined as the last evaluation before

the beginning of the first study drug. Summary statistics, including mean absolute change from baseline, will be determined and tabulated for each measure.

Physical Exams
Physical examination findings at baseline by attending physician will be documented based on chart review. Clinically significant changes will be recorded as AEs. Symptoms of interest previously reported with intravenous Vitamin C based on the package insert will be recorded daily, including dizziness, headache, nausea, vomiting, flushing, rash, or diarrhea. Physicians managing COVID-19 subjects will be listed as sub-investigators and amendments may be made to add investigators to protocol in order to limit research activities and conserve personal protective equipment (PPE).

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

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.

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. 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.

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. 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." 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.

In this study, consent will be obtained by the COVID-19 RICVA providers in order to limit risk of exposure to non-essential staff. The providers are listed as sub-investigators in the study and include experts in hospital medicine, infectious disease, and pulmonary/critical care. Providers will be educated on consent process and additional providers may be added to protocol via amendment process. The consent form itself will either kept in the subject's room until discharge from the hospital and a digital photograph may be obtained in order to limit spread of COVID-19. Infectious disease and epidemiology will be consulted and provide protocols for handling consent paperwork, which may be updated by amendment to protocol. If informed consent is obtained via telephone or virtual meeting, then a witness not related to study team will be present to listen to conversation and sign ICF. All study participants who are consented via telephone or virtual meeting will be offered a physical consent form to be signed via fax or mail.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants and IRB. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IRB and/or Food and Drug Administration (FDA).

10.1.3 CONFIDENTIALITY AND PRIVACY

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

All research activities will be conducted in as private a setting as possible.

Representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

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

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored in a secure, electronic database. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Not applicable to study.

10.1.5 SAFETY OVERSIGHT

Safety oversight will monitored by the PI to ensure accurate recording of the data and reporting of any clinically significant AEs to the IRB or appropriate federal authorities.

10.1.6 CLINICAL MONITORING

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

- Monitoring for this study will be performed by an internal monitoring team, who may or may not be part of the study team.
- On site monitoring will occur annually at the time of IRB continuing review and involve random review of certain data to assess for data accuracy, protocol compliance, and deviations.

10.1.7 QUALITY ASSURANCE AND QUALITY CONTROL

The investigational site will provide direct access to all trial related source data/documents, and reports for the purpose of monitoring and auditing by the IRB, and inspection by local and regulatory authorities.

10.1.8 DATA HANDLING AND RECORD KEEPING

A screening log will be created and stored in a password protected document on a secure network. Data will be entered into a password-protected software.

10.1.8.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at VA under the supervision of the site investigators. The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

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

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into secure data collection software with password protection (Microsoft Excel). Clinical data will be entered directly from the source documents.

10.1.8.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 1 year after study completion.

10.1.9 PROTOCOL DEVIATIONS

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

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.1.10 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

10.1.11 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial.

10.2 ABBREVIATIONS

AE	Adverse Event
AKI	Acute Kidney Injury
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ARDS	Acute respiratory distress syndrome
AST	Aspartate aminotransferase
BMI	Body mass index
CFR	Code of Federal Regulations
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	Coronavirus disease
CRF	Case Report Form
DILI	Drug Induced Liver Injury
DRE	Disease-Related Event
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HIPAA	Health Insurance Portability and Accountability Act
HDIVC	High dose intravenous vitamin C
ICH	International Conference on Harmonisation
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISO	International Organization for Standardization
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SOA	Schedule of Activities
SOC	Standard of Care
SOFA	Sequential Organ Failure Assessment
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

10.3 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
2.0	3/26/20	Added background mechanism proposals, edited abbreviations, pregnancy added as inclusion, changed sub-investigators to research-certified	Requested by IRB
3.0	4/2/20	Edits to inclusion criteria, added study safety labs, adjusted PI/sub-I	Requested by IRB
4.0	4/9/20	Additional information to Exclusion #4 and Study Drug Discontinuation	Clarification
5.0	5/21/20	Amended kidney stone history exclusion to within past 12 months, further detailed clinical parameters for adverse event definitions and natural history of COVID-19	Clarification

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