CLINICAL STUDY PROTOCOL

VIRGINIA COMMONWEALTH UNIVERSITY

C. Kenneth and Dianne Wright
Center for Clinical and Translational Research

SAFEty Study of Early Infusion of Vitamin C for Treatment of Novel Coronavirus Acute Lung Injury

(SAFE EVICT CORONA-ALI)

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Synopsis

Study Description

The purpose of this proof-of-concept, phase 2 safety study is to examine the safety profile, and possible therapeutic effects of high-dose, intravenous vitamin C (HDIVC) compared to placebo in hospitalized adult patients with novel Coronavirus COVID-19 and hypoxemia.

Objectives

To assess the safety profile of a 4-day (96-hour) intravenous vitamin C treatment protocol (comprised of four intravenous infusions a day, that is 50 mg/kg every 6 hours) in patients with laboratory-confirmed SARS-CoV-2 infection manifesting COVID-19 (Novel Coronavirus Disease 2019) with hypoxemia. While performing this phase 2 proof-of-concept, safety study, we will explore the following hypotheses:

Endpoint

Primary Endpoint (Safety): HDIVC infusion does not worsen the patients' outcomes, as measured by differences in the mean 9-point (from 0 to 8) World Health Organization (WHO) ordinal scale for disease improvement, between the two populations at 28, 60 and 90 days:

Table 1.

Patient State	Descriptor	Score
Uninfected	No clinical or virologic evidence of infection	0
Ambulatory	No limitation of activities	1
	Limitation of Activities	2
Hospitalized mild disease	Hospitalized, no oxygen therapy	3
	Oxygen by mask or nasal prongs	4
Hospitalized Severe Disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation and mechanical ventilation	6
	Ventilation + additional organ support – vasopressors, renal replacement therapy, ECMO	7
Dead	Death	8

Secondary Endpoint A (Safety): Daily renal safety biomarkers on the day of randomization (Day 0), then day 1-7. These include (1) daily serum oxalate levels, (2) daily urine microscopic analysis for the presence of oxalate stones (3) daily random urine oxalate and 24-hour urine oxalate levels on days 5,7 and 14.

Secondary Endpoint B (Safety): acute kidney injury-free days at day 28, 90 (stemming from daily AKI assessment while patient is in the hospital).

Secondary Endpoint C (Safety): All-cause mortality to day 28, 60 90 (daily assessment).

Secondary Endpoint D: (Exploratory): COVID-19 and sepsis-specific inflammatory marker levels on Day 0, then on day 7. These include (1) ferritin, (2) D-dimer, (3) lactate dehydrogenase (LDH), (4) interleukin-6, and (5) Trough ascorbate plasma concentrations at 24 hours, 48 hours, 96 hours and final plasma concentration assessment at168 hours.

Secondary Endpoint E: (Safety): Proportion of patients alive and free of respiratory failure through 28-days. (Respiratory failure defined as resource utilization requiring at least 1 of the following: Endotracheal intubation and mechanical ventilation, Oxygen delivered by high-flow nasal cannula (heated, humidified, oxygen delivered via reinforced nasal cannula

at flow rates >20L/min with fraction of delivered oxygen ≥0.5), noninvasive positive pressure ventilation, extracorporeal membrane oxygenation).

Secondary Endpoint F: (Safety): Proportion of patients alive and free of invasive mechanical ventilation at 28-days.

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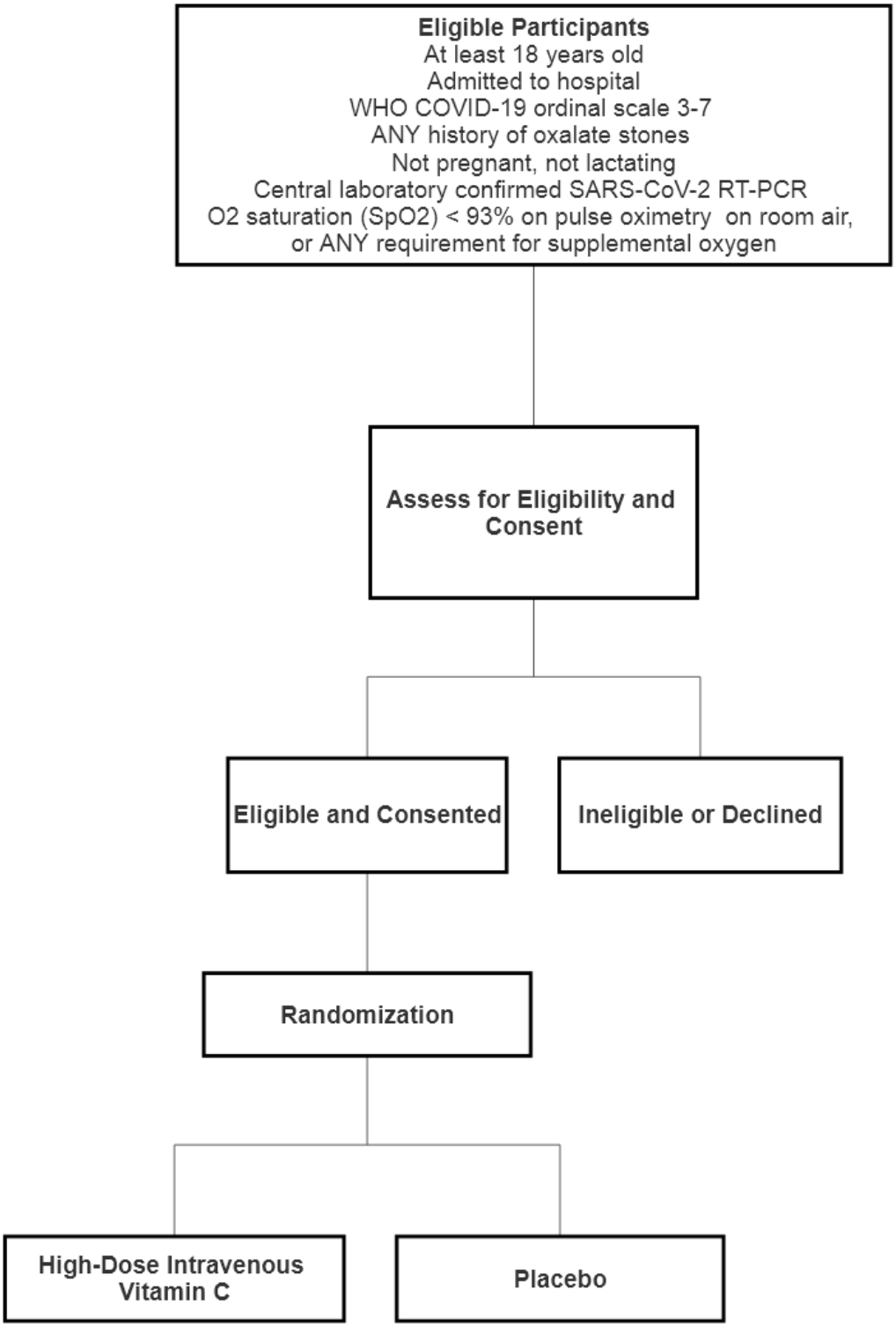
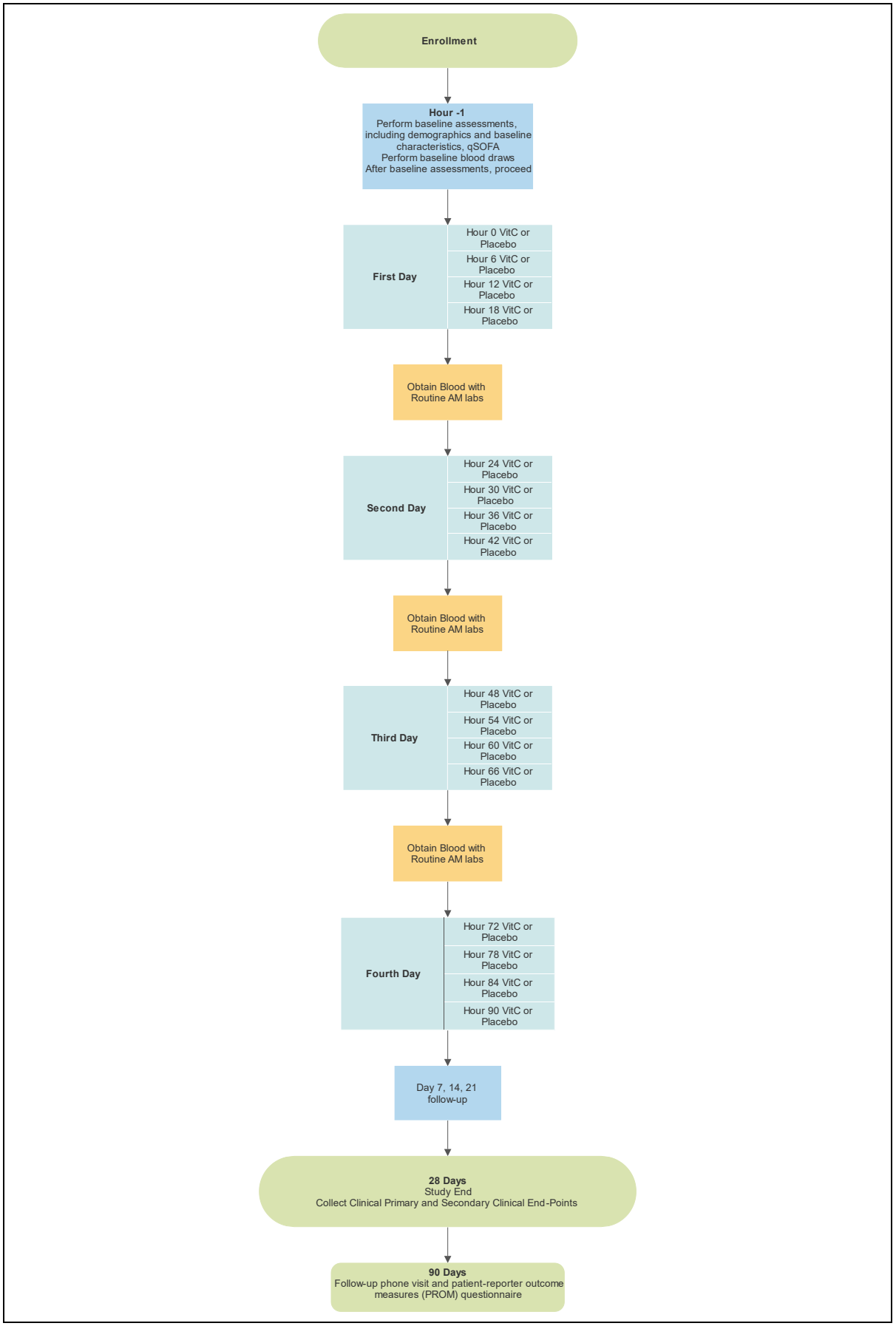
Study Population Adult hospitalized patients who have central laboratory-confirmed COVID-19 (Novel Coronavirus Disease-2019, based on a positive SARS-CoV-2 RT-PCR confirmed within 72 hours before enrollment) and new-onset hypoxemia, defined as oxygen saturation (SpO2) < 93% on pulse oximeter (WHO COVID-19 ordinal scale 3), or requiring any amount of supplemental oxygen (WHO COVID-19 ordinal scale 4-7) at (1) Virginia Commonwealth University Medical Center, and (2) Central Virginia Veterans Affairs (VA) Health System, Richmond, Virginia
Accrual Ceiling The accrual ceiling for this safety trial is a total of 60 patients, between the two centers.
Phase Phase 2, proof-of-concept safety trial.
Description of Sites/Facilities Enrolling Participants The study will involve 2 enrollment sites: (1) Virginia Commonwealth University Medical Center, and the (2) Central Virginia Veterans Affairs (VA) Health System, Richmond, Virginia.
Description of Study Intervention 1. A two-center, prospective, randomized, **double-blind**, placebo-controlled proof-of-concept safety study. 2. A maximum of 60 patients (30 in the treatment, and 30 in the placebo arm) will be enrolled between the two centers. 3. Participants will be randomized to receive either intravenous vitamin C infusion (mixed in 5% dextrose in water) or placebo infusion (5% dextrose in water) in a 1:1 manner. 4. All study drug doses will be administered via an intravenous central or peripheral line infusion. Should no central or peripheral line be available at the scheduled time of infusion, a call should be placed to the pharmacy to determine if the study drug may be co-infused into the line that is infusing a different drug. If co-infusion of study drug is contraindicated, then a maximum of 8 hours may delay study drug infusion. If clinical drug administration schedule is such that study drug will not have an available administration time beyond this delay, a dedicated new intravenous line (peripheral) should be inserted if the patient is agreeable. 5. The study drug will be double-blinded with an identical appearing placebo. 6. Active treatment duration will continue for 96 hours, or until study withdrawal.
Study Duration The study will be completed in two phases: Phase 1 will end on day 28, and phase 2 at 90 day follow up.
Participant Duration The participant duration will be 90 days. Participants will receive the 4-day treatment period (96-hour); if needed, the last study-specific blood work will be collected up to 7 days follow-up after enrollment. Data up to 28 days follow-up will be collected from medical records, standard of care procedures, and chart review. Follow up data and patient-reported outcome measures will be collected by phone visit at day 90.

Schema


Eligible Participants
At least 18 years old
Admitted to hospital
WHO COVID-19 ordinal scale 3-7
ANY history of oxalate stones
Not pregnant, not lactating
Central laboratory confirmed SARS-CoV-2 RT-PCR
O2 saturation (SpO2) < 93% on pulse oximetry on room air,
or ANY requirement for supplemental oxygen
Assess for Eligibility and Consent
Eligible and Consented
Ineligible or Declined
Randomization
High-Dose Intravenous Vitamin C
Placebo


Enrollment
Hour -1
Perform baseline assessments, including demographics and baseline characteristics, qSOFA
Perform baseline blood draws
After baseline assessments, proceed
First Day
Hour 0 VitC or Placebo
Hour 6 VitC or Placebo
Hour 12 VitC or Placebo
Hour 18 VitC or Placebo
Obtain Blood with Routine AM labs
Second Day
Hour 24 VitC or Placebo
Hour 30 VitC or Placebo
Hour 36 VitC or Placebo
Hour 42 VitC or Placebo
Obtain Blood with Routine AM labs
Third Day
Hour 48 VitC or Placebo
Hour 54 VitC or Placebo
Hour 60 VitC or Placebo
Hour 66 VitC or Placebo
Obtain Blood with Routine AM labs
Fourth Day
Hour 72 VitC or Placebo
Hour 78 VitC or Placebo
Hour 84 VitC or Placebo
Hour 90 VitC or Placebo
Day 7, 14, 21 follow-up
28 Days
Study End
Collect Clinical Primary and Secondary Clinical End-Points
90 Days
Follow-up phone visit and patient-reporter outcome measures (PROM) questionnaire

Schedule of Activities (SoA)

Assessments	Day 0-1	Day 1-2	Day 2-3	Day 3-4	Day 5-7**	Day 8-14**	Day 15-21**	Day 22-28**	Day 60& 90
WHO-COVID-19 SCALE	R	R	R	R	R	R*	R*	R*	
AKI, present and stage of AKI	R	R	R	R	R	R*	R*	R*	
Creatinine, serum (BMP also includes sodium, chloride, potassium, BUN, bicarbonate, glucose)	R	R	R	R	R	R*	R*	R*	
Fluid Balance and Urine Output	R	R	R	R	R	R*	R*	R*	
Microscopic urine analysis	R	R	R	R	R	R14[AKI]	R21[AKI]	R28[AKI]	
Serum oxalate	R	R	R	R	R	R14[AKI]	R21[AKI]	R28[AKI]	
Random urine oxalate	R	R	R	R	R				
24-hour urine oxalate collection					R5, R7	R14	R21[AKI]	R28[AKI]	
Alive and Free of ANY Respiratory Failure (yes/no)	R	R	R	R	R	R	R	R	
Alive and Free from INVASIVE mechanical ventilation (yes/no)	R	R	R	R	R	R	R	R	
Oxygen Device **Oxygen Flow** **Air Flow [HHFNC only]** **FiO2 [if not NC]** **Intubated last 24 hours?** ***Date & time of last intubation*** **Extubated last 24 hours or last week?** ***Date & time of last Extubation*** ***ECMO***	S	S	S	S	S	S	S	S	
Body Weight (kg)	S	S	S	S	S	S	S	S	

Vital Signs, Mental Status, qSOFA	S	S	S	S	S	S	S	S	
Maximum norepinephrine rate today	S	S	S	S	S	S	S	S	
Maximum vasopressin rate today	S	S	S	S	S	S	S	S	
Maximum dobutamine rate today	S	S	S	S	S	S	S	S	
Had Cardiac Arrest today (or last week)?	S	S	S	S	S	S	S	S	
Demographics **Comorbidities** **Active Diagnoses** **Admission APR-DRG** **On O2 at home Y/N** ***O2 liters at home*** **Baseline creatinine** **Baseline CKD stage**	S								
Received Dialysis last 24 hours or last week **Received CRRT last 24 hours or last week**	S	S	S	S	S	S	S	S	
Any episode of hypoglycemia last 24hours and week **[Glucose < 50]**	S	S	S	S	S	S	S	S	
Any clinical symptoms of kidney stones? ***Back pain, Flank pain*** ***Hematuria*** ***Visible stone passed*** ***Imaging consistent with new kidney stone***	R	R	R	R	R	R	R	R	
Today's CBC, Differential	R	R	R	R	R	R	R	R	

	including lymphocyte ratio, including potassium, magnesium and phosphorus, LDH, D-Dimer, CRP, Ferritin, ABG, Urinalysis all if performed for patient care, pregnancy test (if applies and was not obtained)									
	Bilirubin Total, AST, ALT, ALP, albumin	**R**				**R7**	**S**	**S**	**S**	
	Received Steroids last 24 hours or last week? **Steroid type and daily cumulative dose [for daily assessment]**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	
	COVID-19 specific treatments, anticoagulants, and dosage: ***Remdesivir*** ***Dexamethasone*** ***Anticoagulants*** ***Other COVID-19 treatments***	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	
	Any atrial arrhythmias over the last interval [afib, aflutter, SVT]	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	
	12-Lead EKG	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
	Any significant ventricular arrhythmias over the last interval [not PVCs]	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	
	Chest X-Ray **Chest Computer Tomography**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
	24-hour urine oxalate on day 5 (after completion of the last dose)	**S**	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**S**
	Lowest daily blood glucose **Highest lactate dehydrogenase**	**RS**	**R**	**R**	**R**	**RS**				

	(LDH), serum Highest D-dimer, plasma Highest ferritin, plasma Highest C-reactive protein (CRP), plasma IL-6, plasma									
	Baseline and Trough Ascorbic Acid (vitamin C levels)	R	R	R	R	R7				
	AKI free-days days Urinalysis evaluation for stones	S	S	RS	S	S	S	S	S	S
	Is the patient still hospitalized? If not Discharge date time								R	
	Total Ventilator-Free Days								R	
	Was the patient paralyzed for ARDS at any point?								R	
	Was placed on iNO at any point?								R	
	Was placed in a prone position at any point?								R	
	Was placed on ECMO at any point during this hospitalization? [VA or VV]								R	
	Did the patient had a tracheostomy at any point in this hospitalization?								R	R
	Date and Time of Death if applies								R	R
	Cause of Death if applies								R	R
	DNR? If yes when was DNR first placed								R	R
	Phone clinic follow-up patient-reported outcome measure									R

PROMIS Dyspnea Assistive Devices and Resources score (version 1.0).									

Footnotes

The study protocol does not include phlebotomy (new stick) just for study labs for days 1-4. If labs are obtained for any other reason, then study labs marked as "R" will be collected as "add-on" to the hospital lab. The protocol does include study labs that will be used specifically for Research marked as RS.

S = standard of care (when feasible), R = research (optional), RS: research specific including phlebotomy; BMP = basic metabolic profile, DNR = do not resuscitate, ECMO = Extracorporeal membrane oxygenation, iNO = inhaled nitric oxide, AKI = acute kidney injury, CRRT = Continuous renal replacement therapy (continuous dialysis), HD = Hemodialysis, CKD = Chronic kidney disease, CRP = C Reactive Protein, ABG = Arterial blood gas, CAM-ICU = ICU Delirium scale, IL-6 = Interleukin 6, afib = atrial fibrillation, aflutter = atrial flutter, SVT = Supraventricular Tachycardia, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase.

R* IF the patient is in the hospital
R7 only on day 7 within that interval
R14 only on day 14 within that interval
R21 only on day 21 within that interval
R28 only on day 28 within that interval
$R14^{AKI}$ collect on day 14 if patient develops AKI and still in the hospital
$R21^{AKI}$ collect on day 21 if patient develops AKI and still in the hospital
$R28^{AKI}$ collect on day 28 if patient develops AKI and still in the hospital

End of Study Definition

Treatment protocol will continue for 4 days (96 hours), and, if needed, the last study-specific bloodwork with being collected on day 7. Study will end if patient is withdrawn from the study, or lost to follow-up. All subjects will be followed to day 28 for collection of clinical outcomes data through electronic health records (EHR) even though the treatment protocol will be completed by 96 hours from randomization at the latest. Secondary outcome data will be collected either during in-person (clinic) visit or via telephone at the 90-day follow-up. **Only acceptable reasons for early study termination include consent withdrawal or loss to follow-up.**

Abbreviations

Abbreviation	Explanation
AE	Adverse Event
AKI	Acute Kidney Injury
ARDS	Acute Respiratory Distress Syndrome
Case Report Form	Case Report Form
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	Coronavirus Disease 2019
D5W	Dextrose 5% Water
DHHS	Department of Health and Human Services
DRE	Disease-Related Event
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Form
ED	Emergency Department
FIP	Feces-Induced Peritonitis
HDIVC	High-Dose Intravenous Vitamin C

ICU	Intensive Care Unit
IND	Investigational New Drug Application
IRB	Institutional Review Board
IV	Intravenous
LDH	Lactic Dehydrogenase
MCV	Medical College of Virginia
RICVA	Central Virginia VA Health System in Richmond, Virginia
SAE	Serious Adverse Event
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SNS	Strategic National Stockpile
SOA	Schedule of Activities
US	United States
VA	Veterans Affairs
VCU	Virginia Commonwealth University

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1 - Statement of Compliance

1.1 Statement of Compliance

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable United States (U.S.) Code of Federal Regulations (CFR). The Principal Investigator will assure that no deviation from funding agency and documented approval from the Institutional Review Board (IRB) will occur 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.

2 - Introduction

2.1 Study Rationale

The World Health Organization (WHO) has declared COVID-19, the syndrome caused by the Severe Acute Respiratory Syndrome novel Coronavirus 2 (SARS-Cov-2), a pandemic. (1) As of April 23, 2020, there are 2,658,062 cases of COVID-19, with 184,643 deaths. (2) An alarming report (3) suggested that an unmitigated epidemic will cause up to 240,000 deaths in the United States alone. So far, no reliable treatment has been found, and the most promising measures have been the non-pharmacologic measures of social distancing, handwashing, school closures, and case isolations, among others. (3)

A significant area of concern, and a potential contributing factor to the number of deaths, has been the effect of the SARS-Cov-2 on Intensive Care Unit (ICU) bed availability. Worst-case scenario analyses suggest that in the United States, 1.9 million COVID-19 patients could be admitted to the ICU, and 50% of those (960,000) would require invasive mechanical ventilation. (5) The obvious problem is that the U.S. has approximately 200,000 mechanical ventilators available, *including* the Centers for Disease Control (CDC) Strategic National Stockpile (SNS), the addition of anesthesia machines and old, retired stockpiled ventilators. (4) Also, 48% of U.S. hospitals have no intensive care unit specialists. (6)

The most common reason for ICU admission for COVID-19 patients is a respiratory failure caused by SARS-Cov-2 induced Sepsis and Acute Respiratory Distress Syndrome (ARDS). (4) While the need for a treatment for COVID-19 is urgent and the non-pharmacologic efforts to "flatten the curve" will play a significant role in reducing the burden on the US health care system, being able to slow or stop the incidents of SARS-Cov-2 induced ARDS would further reduce the pressure on health care systems. Furthermore, the success of efforts to lift the current extreme restrictions, before herd immunity is achieved or a vaccine is available, would be bolstered by having a safe, easy to implement, and effective intervention aimed at reducing the progression of conditions which lead to a high need for ICU beds and ventilators. This study proposes to use High Dose Intravenous Vitamin C (HDIVC) to stop the progression of early COVID-19 Acute Lung Injury (ALI) before it progresses to Sepsis, Acute Hypoxemic Respiratory Failure (AHRF) and ARDS. If successful, this would reduce the number of COVID-19 patients requiring endotracheal intubation, mechanical ventilation, ICU admission, and potentially offer public health benefits.

2.2 Background

In December 2019, a cluster of pneumonia cases was reported in Wuhan, China, and a novel coronavirus, SARS-Cov-2, was identified. (7) The World Health Organization (WHO) designated the respiratory disease as coronavirus disease (COVID-19) on February 12, 2020. Numerous reports have since characterized the COVID-19 clinical syndrome, ranging from asymptomatic/mild illness to severe disease leading to respiratory failure requiring mechanical ventilation (i.e., ARDS, multi-organ failure, sepsis, and death). (7 – 9) A safe, effective, and inexpensive therapy is urgently needed, one that can alter the natural course of the disease process and reduce strain on healthcare systems. High Dose Intravenous Vitamin C (HDIVC) is one such potential therapy.

Preliminary studies suggest that HDIVC is a safe and possibly effective treatment for ARDS, including ARDS of viral origin. (10) Since 1986, there has been increasing evidence that HDIVC may be useful in preventing multi-organ failure and mortality in ARDS patients. It remains unknown if HDIVC given early can halt the progression to ARDS and if it is effective in avoiding COVID-19 ARDS.

Basic Science Data to Support the Use of HDIVC in ARDS

Animal studies performed at VCU have shown that vitamin C in high doses prevents pulmonary neutrophil infiltration in murine feces-induced peritonitis (FIP) model of sepsis/ARDS (Figure 1). Vitamin C (AscA) high parenteral doses increased the clearance of bacteria from the circulation in a preclinical model of ARDS. In the same murine model of sepsis, blood was obtained using sterile technique at varying time points after induction of feces induced peritonitis with or without associated HDIVC administration. Ten µl of blood was plated on agar plates and colonies enumerated following 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 increased alveolar protein content after induction of FIP with unchanged alveolar protein content in FIP animals treated with HDIVC (data not shown), indicating that HDIVC maintained lung barrier function between the blood and the airspace. (25)

Human Trials for the use of HDIVC in Sepsis-induced ARDS

Vitamin C supplementation has been given in numerous trials for respiratory disease with mixed results (22,23).

The first study, published in 1986, compared 16 ARDS patients treated with intravenous

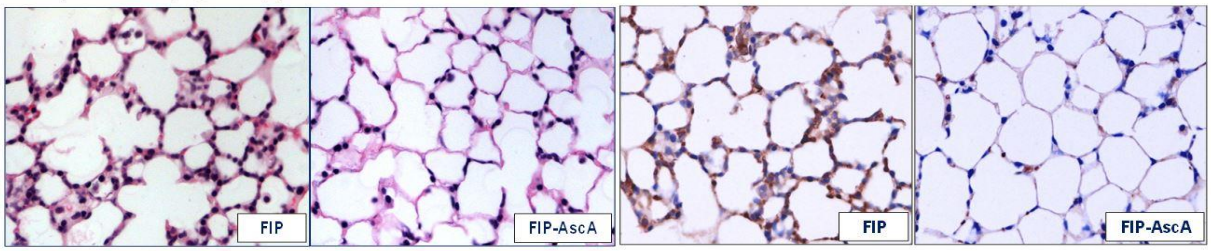


Figure 1. Lung pathology slides (40x magnification) of **septic** mice with fecal induced peritonitis (FIP) demonstrating Acute Lung Injury (ALI) a feature of ARDS. On the right, sections of lung (40x magnification) 16 hours after infusion of Ascorbic Acid (FIP-AscA), shows resolution of inflammation, and attenuated inflammatory cell sequestration.

vitamin C (1000 mg IV every 6 hours) plus antioxidants (N-acetylcysteine, selenium, and vitamin E) to 16 ARDS patients who received the standard care at that time (i.e., control group). There was a dramatic reduction in mortality in the vitamin C group versus the standard of care group: 37% versus 71% ($p<0.01$). (12) A phase I trial in 2014 showed that plasma vitamin C levels in patients with severe sepsis and ARDS were low, almost at scorbutic levels (13) and that HDIVC administration had a dose-dependent effect in the prevention of multi-organ failure, as measured by the Sequential Organ Failure Assessment (SOFA) scores. (14) Patients who received a total of 200 mg/kg/day of HDIVC for 4 days (administered in 50 mg/kg/dose, every 6 hours) had significantly lower organ failure scores than patients in the placebo group. In addition, the patients in the HDIVC group (200 mg/kg/day) recovered from the scorbutic plasma levels of vitamin C, achieving plasma levels as high as 3,000 µM on day 4.

In one study with marine recruits (n=674), a randomized, double-blind study of oral vitamin C at 2,000 mg/day reduced the reported pneumonia cases compared with placebo

. High dose vitamin C has also been administered in a case of Enterovirus-induced ARDS in a patient who developed severe respiratory failure and required mechanical ventilation and extracorporeal membrane oxygenation (ECMO) support. Rapid improvement in oxygenation occurred early after HDIVC, which was administered for 7 days total. The patient experienced no adverse events from HDIVC or any long-term sequelae. (21)

A recent trial using the trio of vitamin C/thiamine/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 we are proposing, was an open label, unblinded, and not in patients with ARDS. (15)

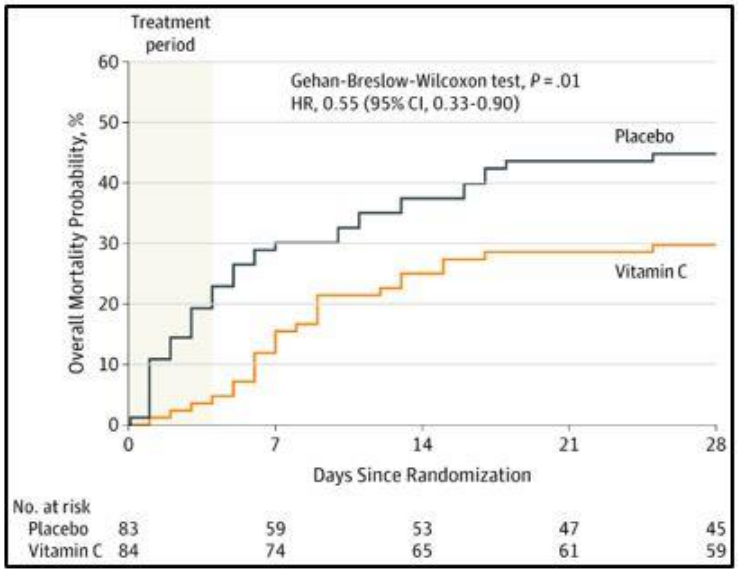
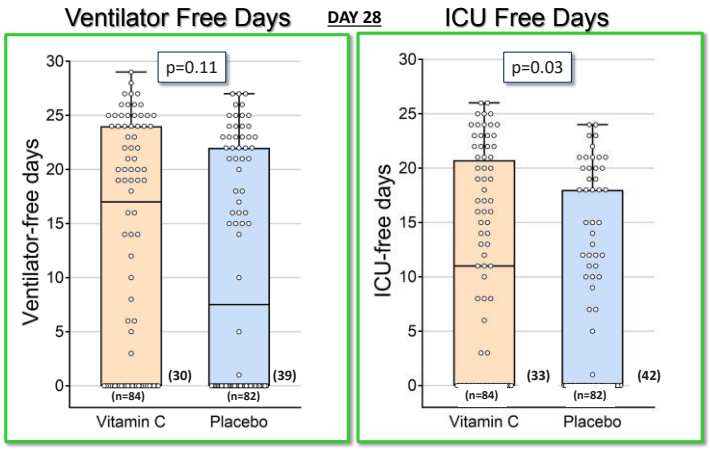


Figure 2. CITRIS-ALI 28 day all-cause mortality

The multicenter, randomized, double-blind CITRIS-ALI trial led by our lab enrolled 167 patients with ARDS who were randomized to receive 50 mg/kg of HDIVC or a placebo every 6 hours for 4 days. The treatment group showed a statistically significant difference in 28-day all-cause mortality compared to the placebo group (Figure 2), although this was a secondary outcome. (11) The 28-day mortality was 29.8% in the vitamin C group versus 46.3% in the placebo group. This can be translated as a Number Needed to Treat (NNT) of 6 patients to save one life from ARDS. The statistical effect on mortality remained up to 60-days following trial completion. (11)



Fowler et al. JAMA. 2019 PMID: 31573637

Figure 3. Ventilator-free days

In addition, the CITRIS-ALI trial (11) showed that ARDS patients who received HDIVC (at 200 mg/kg/day for 4 days, n=84) had higher ventilator-free days compared to placebo (n=83), at 13.1 days compared to 10.6 days (mean difference, 2.47: 95% CI -0.9-5.85, P=0.15). Patients treated with HDIVC also had a significant increase in ICU free days (10.7 in the vitamin C group vs. 7.7 in the placebo group (mean difference, 3.2; 95% CI, 0.3 to 5.9; $P = .03$).

It is important to note that none of the participants in the study developed any significant side effects from the intravenous vitamin C. The CITRIS-ALI trial results show promise for the use of HDIVC for acute respiratory failure with few significant short- or long-term side effects.

Data for the use of HDIVC in COVID-19 induced Sepsis and ARDS

High-dose Intravenous Vitamin C (HDIVC), even at much higher doses than we are suggesting in the present protocol, has been used successfully to treat COVID-19 patients in China. (18) The doses varied between 10,000 and 20,000 mg per day. (18) The authors report

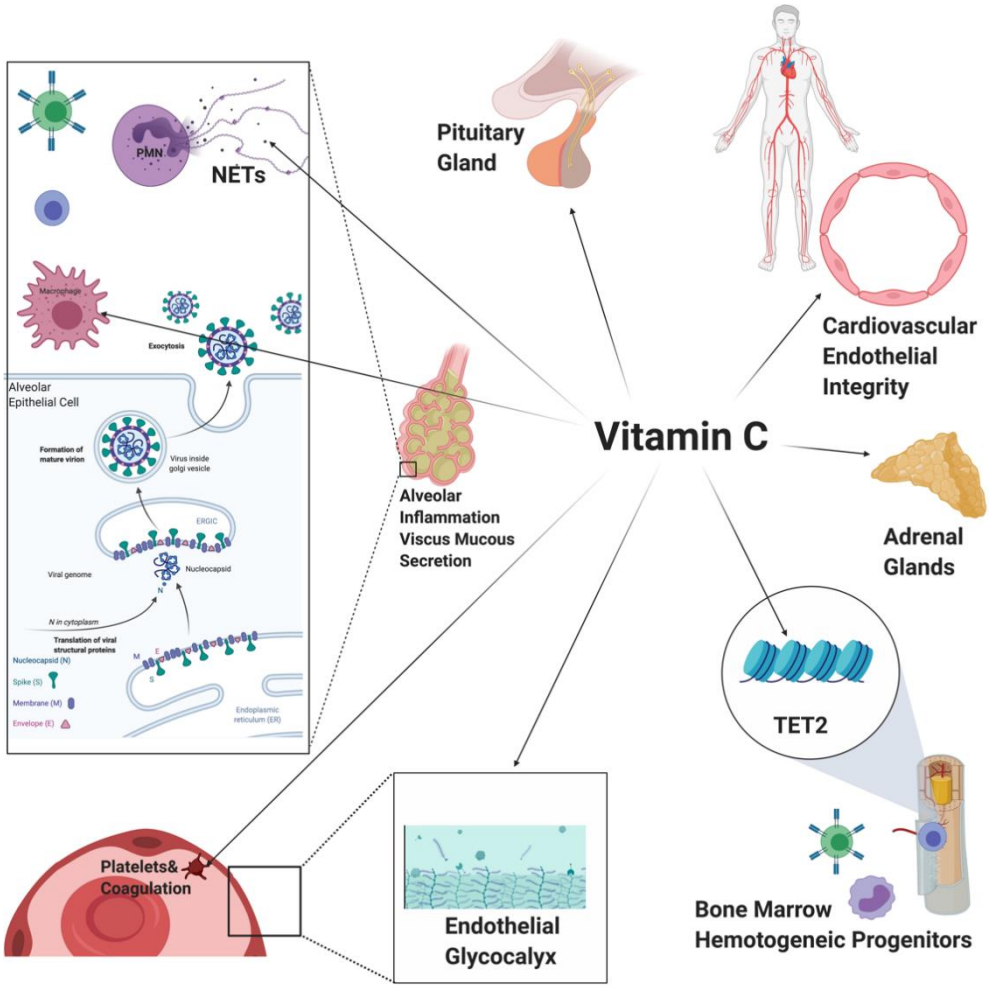


Figure 3: Known biological effects of Vitamin C and suggested effects on COVID-19. NET: Neutrophil Extracellular Traps, TET-2 (tet methylcytosine dioxygenase 2). The methylcytosine dioxygenase that catalyzes the conversion of methylcytosine to 5-hydroxymethylcytosine. The encoded protein is involved in myelopoiesis, and defects in this gene have been associated with several myeloproliferative disorders. Vitamin C is a cofactor for TET2. This figure was created by the authors of the protocol for the purpose of the protocol (unpublished data), based

that the oxygenation index improved in all these cases in "real-time," and all the patients were cured and discharged. Importantly, none of the patients experienced any significant side effects. (18) A randomized clinical trial is underway in Wuhan, China, to administer 12,000 mg of intravenous vitamin C every 12 hours for 7 days (significantly higher than our suggested protocol) compared with placebo. (NCT04264533) Further trials are currently in the planning and enrolling phase for HDIVC in sepsis and ARDS. Consumer groups are petitioning and urging the FDA to approve high-dose intravenous vitamin C for the COVID-19. (20) This petition has reached 17,700 signatures as of May 5th, 2020.
A summary of possible mechanisms of HDIVC for SARS-CoV-2 infection based on existing knowledge is presented in figure 3 below.

How does Vitamin C improve ARDS and Respiratory Failure in Humans?

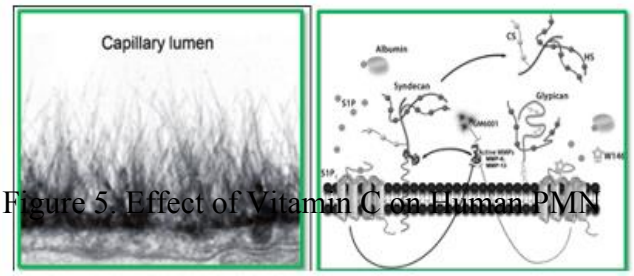


Figure 4. The endothelial glycocalyx

The endothelial glycocalyx (Figure 4) plays an essential vasculo-protective role, modulating permeability, blood cell/vessel wall interactions, and shear stress sensing. Current research indicates the glycocalyx modulates inflammation and maintains anti-thrombogenic, anti-adhesive endothelial surfaces. Glycocalyx components such as syndecan-1 are shed by enzymatic digestion (e.g., metalloproteinases) and exposure to reactive oxygen intermediates as occurs with acute inflammation of sepsis. Enzymatic degradation and release of cell-free DNA from activated neutrophils leads to loss of glycocalyx components (e.g., syndecan-1), which in turn leads to the display of potent adhesion receptors. This results in the sequestration of activated neutrophils and platelets in microvascular spaces amplifying vascular injury. In lung, this process produces loss of pulmonary capillary barrier function and rapid onset of alveolar injury with subsequent flooding of the alveolar space.

Figure 5 shows that neutrophils activated in vitro release cell-free DNA (Panel B). Cell-free DNA release from activated neutrophils treated with vitamin C is *completely attenuated*. (25)

Figure 5. Effect of Vitamin C on Human PMN

In a post-hoc analysis, we used plasma obtained from patients enrolled in the multicenter, double-blind, randomized, placebo-controlled CITRIS-ALI trial. (27) Figure 6 shows that patients who received intravenous vitamin C exhibited significantly decreased 48-hour plasma cell-free DNA levels compared to placebo, and, importantly, vitamin C infusion was associated with an attenuated plasma syndecan-1 levels compared to placebo (unpublished observation). These data from CITRIS-ALI in a well-characterized patient population with sepsis-induced acute respiratory distress syndrome (ARDS) shows that *increased concentrations of plasma cell-free DNA is associated with increased plasma syndecan-1 levels*.

We further found that changes in both 48-hour cell-free DNA and 48-hour syndecan-1 levels predicted 28-day all-cause hospital mortality (Figure 7). In this analysis, increased syndecan-1 measured at 48 hours highly correlated with worsened indices of oxygenation (data not shown). Degradation of syndecan-1 plays a vital role in early phase vascular injury, serving as a risk factor for plasma leakage. These findings have implications. Plasma syndecan-1 measurement in septic virally infected patients may serve as a critical biomarker and possibly

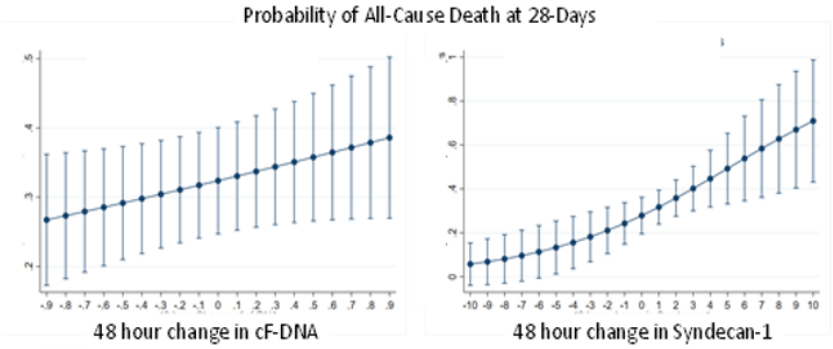


Figure 7. Human mortality, cf-DNA and syndecan-1

convey important prognostic implications of organ dysfunction produced by COVID-19 infection. Suwarto et al. found that systemic viral infection (i.e., dengue) is associated with substantial endothelial glycocalyx degradation, as evidenced by significant increases in plasma syndecan-1. (26)

These results suggest that patients could benefit from therapies targeted at protecting or restoring the glycocalyx. The data presented here strongly suggest that vitamin C's protection of the glycocalyx in septic ARDS patients led to the reduced extent of organ failure observed in CITRIS-ALI. (27) (Figure 8). The mechanisms that degrade the glycocalyx are still not fully clarified. Still, the results of our biomarker analysis reported here more visibly point to neutrophil extracellular trap formation (i.e., cell-free DNA) as an essential factor in glycocalyx degradation. Efforts to restore the glycocalyx likely will include the stabilization of endothelial cell surface syndecan-1 expression, a vital backbone of the glycocalyx. The results of our biomarker analysis in patients from the CITRIS-ALI trial lead us to reason that intravenous infusion of vitamin C may well occupy a future role in glycocalyx protection and restoration and may have a substantial role in COVID-19 infection. With the available evidence pointing to vitamin C as playing a role in glycocalyx protection, likely vitamin C's capacity both as an antioxidant and an anti-inflammatory agent is critical.

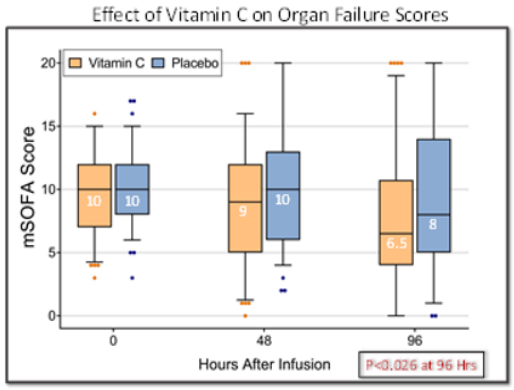


Figure 8. Vitamin C, cfDNA and syndecan-1

Adverse effects of High-Dose Intravenous Vitamin C
An NIH expert panel concluded that "Studies have shown that vitamin C can be safely administered to healthy volunteers or cancer patients at doses up to 1,500 mg/kg", as per High-dose vitamin C (PDQ®)–Health professional version. (19). No safety issues were reported in the CITRIS-ALI study. (11) Another VCU study examining the use of HDIVC in patients undergoing atrial fibrillation ablation also reported no safety issues. (17)
While vitamin C infused in high concentrations does pose a potential area of concern related to **oxalate formation** and the possibility of oxalate crystal formation in the urine that may lead to renal system stone formation, no stone formation was observed during the CITRIS-ALI trial where vitamin C at 200 mg/kg per day was administered for 4 days. (17) More specifically for COVID-19, in a recent study none of the patients with COVID-19, who received high-dose intravenous vitamin C experienced any significant side effects due to the vitamin C. However, the CITRIS-ALI study did not look specifically for oxalate nephropathy, or long-term kidney outcomes in terms of AKI free-days days (18) In contrast, studies of *other* potential COVID-19 treatments have already been halted due to serious side effects. (16) **There is a clear knowledge gap in the literature in terms of the renal outcomes of high-dose, intravenous vitamin C. The highest level of evidence in terms** of oxalate nephropathy and high-dose intravenous vitamin C is limited to case reports (28-30). A recent systematic review of high-dose intravenous vitamin C, identified "identified 74 relevant articles reporting adverse events of vitamin C, but most studies did not have control or placebo group." (31) There were nine high-quality double-blind randomized control trials (RCTs) reviewed did not show any significant adverse effects comparing to the placebo group. (31) Therefore, there is no definitive evidence that side-effects attributed to vitamin C could be from other therapies. Specific side-effects attributed to high-doses of intravenous vitamin C were " specific adverse events attributed to high-dose IV vitamin C therapy, included "five cases of oxalate nephropathy, five cases of hypernatremia, three cases of hemolysis in patients with G6PD deficiency, two cases of glucometer error, and one case of kidney stones." (31)
Given its success in improving outcomes for non-COVID-19 related ARDS, research suggests that HDIVC given early may also prevent the progression to ARDS in COVID-19. While treatment of COVID-19 would take priority and would not be withheld from patients with ARDS, HDIVC may provide an important therapy to reduce the burden on the US health care system. **Therefore, a proof-of-concept, phase 2 safety trial is needed.**

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

Potential Physical Risks of Ascorbic Acid Infusion: dry mouth, nausea, vomiting, dizziness, headache, factitious hyperglycemia (if checked with a point of care (POC) glucose testing), oxalate nephropathy, crystalluria, kidney stones and hemolysis in people with G6PD.
Potential Psychological, Social, and Legal Risks of Ascorbic Acid Infusion: No psychological, social or legal risks are identifiable from an extensive literature search.
Risks of Blood Draws: Most research data will be collected via standard of care blood draws; however, it may be necessary to do study specific blood draws when participants are enrolled and on study day 7. The risks involved in drawing blood from a vein may include, but are not limited to, momentary pain at the site of the blood draw, possible bruising, redness, and swelling around the site, bleeding at the site, feeling of lightheadedness, and very rarely, an infection at the site of the blood draw.
Since this is an ICU study, blood draws are required only if the patient does not already have an arterial or central line. If patient has one of these lines, then blood draws can be obtained through these lines without sticks. **All effort is done for all study labs to be done with AM labs and other labs that are being done for clinical care. Usually ICU patients get many**

blood draws throughout the day for other reasons. The consent 1.83 includes that patients may need up to 20 blood draws additional which will **replace the POCT glucose measurements, since POCT glucose is not reliable. Further this need to continue up to 72 hours after the last infusion to ensure that glucose measurements are accurate and minimize the risk of iatrogenic hypoglycemia.**
Risks of Peripheral Intravenous (IV) Catheter insertion: The vast majority of inpatients already have at least one peripheral IV line. In the rare occasion that an IV is not present, the study intervention requires infusion through a peripheral IV. Risk of peripheral IV insertion are exactly the same as the risk of blood draws. Risks are generally rare, since this procedure is performed hundreds of times a day in our hospital on all patients. Risk include insertion site pain, phlebitis, hematoma formation, and infusate extravasation. There are also extremely rare, but significant complications have been reported, including bloodstream and local infections, air embolization, nerve damage, arterial puncture. It is worth noting that when used as part of standard of care, peripheral IVs are inserted by the bedside nurse with just verbal consent.

Glucose Monitoring Plan:
Guidance for blood glucose monitoring in patients enrolled in the SAFE-EVICT-CORONA-ALI Trial:
Ascorbic acid is known to artifactually raise point of care (POC) blood glucose readings by all POC devices. Thus, extreme care must be taken to assure an accurate blood glucose level from a metabolic laboratory (single serum glucose, SSG) before initiating insulin therapy, including sliding scale or scheduled insulin.
Critical Care and Inpatient Unit Nursing and Treating Physician must be informed of vitamin C's effect on POC blood glucose and arterial blood gas measured glucose values. The PI(s) will instruct the ordering providers how to monitor blood glucose and insulin management in the study. Study personnel will follow each study patient closely to monitor insulin use to ensure that POC glucose screening is suspended for the research subject during the 96-hour treatment period. The following guidance for blood glucose monitoring in patients enrolled in this study will be provided to the attending staff:

- This patient is enrolled in a study with Vitamin C, which artefactually increases POC glucose testing.
- Special Order set should be used with monitoring of blood glucose.
- Use only central laboratory serum glucose quantification method via **BMP utilizing the hexokinase method and calibrated and accredited hospital analyzers**.
- Do Not Initiate or Utilize Sliding Scale, Scheduled Insulin, or Continuous Insulin Infusion without Laboratory Confirmation of Blood Glucose through the central laboratory **chemistry with BMP (hexokinase method).**
- Those receiving insulin infusion or sliding scale insulin as a part of the standard of care should 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 central laboratory metabolic blood glucose results, whether or not the study patient is receiving insulin.
- **Do not use any POC device for glucose monitoring**. POC glucose testing may resume 36 hours after the last infusion of study drug.

2.3.2 Known Potential Benefits
The first study, published in 1986, compared 16 ARDS patients treated with intravenous vitamin C (1000 mg intravenous every 6 hours) plus antioxidants (N-acetylcysteine, selenium, and vitamin E) to 16 ARDS patients who received the standard care at that time (i.e., control group). There was a significant reduction in mortality in the vitamin C group: 37% versus 71% in the standard care group ($p<0.01$). (12) A phase I trial in 2014 revealed that plasma vitamin C levels in patients with severe sepsis and *ARDS were low, almost at scorbutic levels* (13). HDIVC administration (200 mg/kg/day) raised plasma levels up to 3,000 μM by day 4 and had a dose-dependent effect in the prevention of multi-organ failure, as measured by the Sequential Organ Failure Assessment (SOFA) scores. (14) As mentioned above, our Phase 2 double-blind, placebo-control multicenter randomized control trial (CITRIS-ALI, 11) also suggested that 200mg/kg/day of HDIVC (administered in 50 mg/kg/dose, every 6 hours) reduces the extent of organ failure (figure) and reduces 28-day and 60-day mortality in ARDS. Finally, ongoing treatment in China suggests that HDIVC may be effective in preventing ARDS in patients with COVID-19. The results of CITRIS-ALI suggested that the **Number Needed to Treat (NNT) is approximately six to save on human life.**

2.3.3 Assessment of Potential Risks and Benefits
The identifiable risks arising from exposure to the intravenous ascorbic acid infusion are low. Due to the severity of COVID-19, the study related risks associated with the study procedures

are not greater than the risks associated with hospitalization due to COVID-19. Given the low risk associated with the study 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

3.1 Objectives and Endpoints

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
WHO COVID-19 9-point Ordinal Scale Outcome	MEAN SCORE ON DAY 28, 60 AND 90	This ordinal scale captures a range of safety outcomes and is ideal for safety studies. If any other unanticipated clinical harms of high-dose intravenous vitamin C occur, will be captured on this outcome.
Secondary		
Renal safety biomarkers	Daily serum oxalate Daily urinalysis 24-hour urine oxalate on day 5	Measured biomarkers will provide a sensitive way to identify the mechanisms of oxalate nephropathy
AKI-free days at day 28 and 90	Renal-failure free days, with AKI defined by the KDIGO criteria (see table below).	This endpoint aims to identify long-term kidney outcomes among the two groups
All-cause mortality at 28 60 and 90 days	Safety Endpoint	If the therapy offers significant benefit, or harm, compared with placebo
Exploratory biomarkers	Ferritin, D-dimer, LDH, CRP, plasma IL-6 on day 7	The biomarkers aim to help interpret the outcomes, and further explain clinical differences in the above-measured outcomes
Ascorbate trough levels	Plasma ascorbate at baseline (0 hours) levels at 24 hours, 48 hours, 96 hours and 168 hours	The PK of ascorbate is still unknown in the target patient population
Alive and free of significant respiratory failure through 28-days	At 28-days is the patient alive and not in respiratory failure, the principal cause of death by COVID-19	Respiratory failure defined as at least one of the following: • Endotracheal intubation and mechanical ventilation • •Oxygen delivered by high-flow nasal

		cannula (heated, humidified) • Oxygen delivered via reinforced nasal cannula at flow rates >20L/min with fraction of delivered oxygen ≥0.5) • Noninvasive positive pressure ventilation (CPAP or BIPAP) • Extracorporeal membrane oxygenation (ECMO of any type)
Alive and free of mechanical ventilation at 28-days	Proportion of patients alive and free of invasive mechanical ventilation at 28-days.	Is the patient dependent on mechanical ventilation by 28-days, that is development of significant chronic respiratory failure

WHO COVID-19, 9-point Ordinal Outcome Scale		
Patient State	Descriptor	Score
Uninfected	No clinical or virologic evidence of infection	0
Ambulatory	No limitation of activities	1
	Limitation of Activities	2
Hospitalized mild disease	Hospitalized, no oxygen therapy	3
	Oxygen by mask or nasal prongs	4
Hospitalized Severe Disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation and mechanical ventilation	6
	Ventilation + additional organ support – vasopressors, renal replacement therapy, ECMO	7
Dead	Death	8

Acute Kidney Injury (AKI) Definitions per KDIGO guidelines

Khwaja, A. (2012). KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clinical Practice*,*120*(4), c179-c184.

Stage	Serum creatinine	Urine output
1	1.5–1.9 times baseline OR ⩾0.3 mg/dl (⩾26.5 μmol/l) increase	<0.5 ml/kg/h for 6–12 hours
2	2.0–2.9 times baseline	<0.5 ml/kg/h for ⩾12 hours
3	3.0 times baseline OR Increase in serum creatinine to ⩾4.0 mg/dl (⩾353.6 μmol/l) OR Initiation of renal replacement therapy OR, In patients <18 years, decrease in eGFR to <35 ml/min per 1.73 m^2	<0.3 ml/kg/h for ⩾24 hours OR Anuria for ⩾12 hours

4 - Study Design

4.1 Overall Design

1. A phase 2, two-center, prospective, randomized, double blind placebo-controlled safety clinical trial.
2. A maximum of 60 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) in a 1:1 manner.
4. Active treatment will continue for 96-hour period or consent withdrawal or loss to follow-up. One-fourth of the 24-hour calculated dosage will be administered **in 30-minute intravenous infusions of 50 mg/kg and occur every 6 hours** for a total of sixteen 30-minute infusions. **That is 200 mg/kg total per day for 4 days.** The standard of care therapy will be followed. All COVID-19 known therapies will be allowed.
5. All participants will be followed for a total of 28 days on the first phase of the trial and at 60 and 90 days on the second phase.

6 All participants, without exception, will receive the standard of care for COVID-19 including the use of remdesivir, dexamethasone, plasma and other, as a component of standard of care for patients hospitalized with severe disease, in settings where remdesivir is available via the emergency use authorization mechanism.

Analysis/Interim Monitoring:

1. **Only reasons for study withdrawal are withdrawal of consent and, or loss to follow-up**
2. The DSMB will review all SAEs in a narrative and qualitative fashion (not only quantitative), to avoid underpowered statistical misconceptions.
3. Protocol compliance will be monitored by the study team daily discussion. 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 (DSMB) to determine if the study should stop for safety reasons.

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. **This study will provide an opportunity to explore specifically the specific safety of high-dose intravenous vitamin c and explore the hypothesis generated by case reports, that high-dose vitamin C has clinically significant adverse effects on the kidneys.** By administering the infusion at the first objective sign of worsening oxygenation, documented by Pulse Oximetry Saturation(SpO2)/Fraction of Inspired Oxygen (FiO2) – that is the 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.

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. (29) Oral doses cannot achieve therapeutic plasma concentrations. (34-35) 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 2 human ARDS (CITRIS-ALI) trial. (11) **The 200 mg/kg/24-hour IV (50 mg/kg IV every 6 hours**) dosing protocol was determined from analysis of the CITRIS-ALI (200mg/kg IV levels, that was 50 mg/kg IV every 6 hours) plasma ascorbate levels. **A special consideration is given for patients who will develop KDIGO definition AKI, stage 2 or 3 after the infusion. In this scenario, the dosage will be reduce by 50%, for each subsequent**

infusion, until the AKI resolves completely. We are using a dosing ceiling of 15 grams per day, for consideration of vitamin C dosages in obese population. Under no circumstance an individual infusion dose will exceed 3.75 grams of vitamin C, or the daily infused dose above 15 grams.

4.4 End of Study Definition

Active treatment period will continue for 4 days, that is 16 infusions of 50 mg/kg intravenous vitamin C, 30-minutes each infusion. The last study-specific bloodwork with be collected at 7 days. **All patients will remain in the study through the end of double-blind period and final study blood collection.** Participation will end if patient withdraws the consent or is lost to follow-up. Clinical data, including a final blood draw, will be collected through day 7. All subjects will be followed to day 28 via chart review for collection of clinical outcomes data. 90-day secondary outcomes will be assessed either during clinic visits or via telephone at 60 and 90 days. **Only acceptable reasons for early study termination include consent withdrawal, or loss to follow-up.**

5 - Study Population

5.1 Inclusion Criteria

- Adults of 18 years or older
- Patients hospitalized with a diagnosis of COVID-19 based on central laboratory-confirmed COVID-19 Novel Coronavirus Disease-2019, based on a positive SARS-CoV-2 RT-PCR confirmed **within 72 hours** prior to enrollment of nasal, oropharyngeal, or BAL specimen with hypoxemia, (i.e., decrease in oxygenation, as outlined below)
- Pulse oximetry saturation (SpO2) < 93% on room air in WHO COVID-19 ordinal scale 3 patients, regardless the need for assisted ventilation, or oxygenation.
- Any new requirement of supplemental oxygen, with any oxygen device (WHO COVID-19 ordinal scale 4-7, regardless of pulse oximetry reading)
- In patients with supplemental oxygen at home, **any increase** in the requirement of supplemental oxygen.
- In ICU level of care

5.2 Exclusion Criteria

- Age less than 18 years
- Known allergy to Vitamin C
- Inability to obtain consent from patient or next of kin
- Presence of diabetic ketoacidosis
- **ANY** history of oxalate stones at any time
- **Patients with Kidney Disease Improving Global Outcomes (KDIGO), CKD stage 4 (eGFR < 30 ml/min, CKD stage 5 and end-stage renal disease on dialysis patients are excluded.**
- **Patients with Acute Kidney Injury, stage 3 (see page 28).**
- Pregnant, or lactating
- Known diagnosis of glucose-6-phosphate dehydrogenase (G6PD) deficiency
- Patients who received the following medications within 7 days prior to enrollment, or plan to receive during enrollment, or 7 days after enrollment: aluminum hydroxide, bortezomib, copper, deferoxamine, amphetamines including derivatives such as fluphenazine.
- Patients with active sickle cell crisis
- Prisoners
- Patients outside ICU level of care.

5.3.1 Special Considerations: Minorities

The study will emphasize recruitment of ethnic minorities and patients over 65. Specifically, a Spanish language consent will be available, and 24-hour real-time translation services. Furthermore, an attempt will be made to obtain consent and agreement with prison warden in the Richmond region. Once approval has been signed from prison leadership and the local IRB, inmates may be enrolled as well.

5.3.2 Special Considerations: Chronic Kidney Disease and Acute Kidney Injury patients

Special consideration will be given to avoid excluding patients with acute kidney injury (AKI). Recent evidence suggests that AKI develops in up to 60% of high-risk COVID-19 patients. (33) The receptor for SARS-CoV- 2 is ACE2 receptor, which is expressed in kidney much more so than in lung. (33) Autopsy studies in COVID-19 demonstrated acute proximal

tubular injury, and peritubular erythrocyte aggregation and glomerular fibrin thrombi with ischemic collapse. (33) Therefore, we have the following provisions:

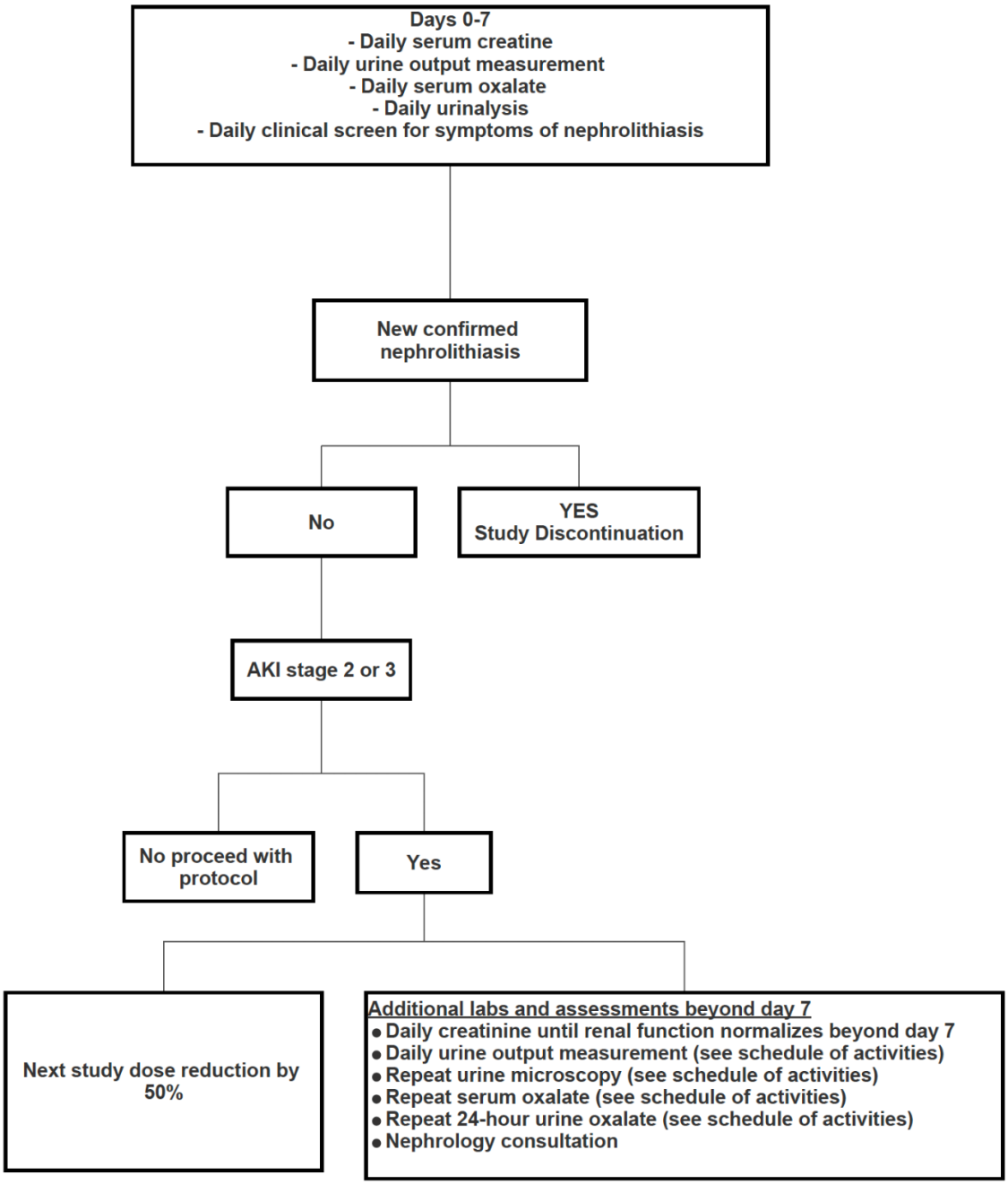
- Exclude patients with KDIGO CKD stage 4 (eGFR < 30 ml/min) or worse. That means patients with KDIGO CKD stage 5 and/or any form of dialysis at baseline are also excluded.
- Exclude patients with pre-existing AKI, stage 3
- Immediate reduction of each infusion dose by 50% if patients develop KDIGO defined AKI stage 2 or higher, until the AKI resolves completely. If the AKI resolves completely, normal dosing can be resumed.
- All AKI and CKD definitions are following the 2012 KDIGO guidelines: Khwaja, A. (2012). KDIGO clinical practice guidelines for acute kidney injury.Nephron Clinical Practice,120(4), c179-c184.

Acute Kidney Injury (AKI) Definitions per KDIGO guidelines

Khwaja, A. (2012). KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clinical Practice*,*120*(4), c179-c184.

Stage	Serum creatinine	Urine output
1	1.5–1.9 times baseline OR ⩾0.3 mg/dl (⩾26.5 μmol/l) increase	<0.5 ml/kg/h for 6–12 hours
2	2.0–2.9 times baseline	<0.5 ml/kg/h for ⩾12 hours
3	3.0 times baseline OR Increase in serum creatinine to ⩾4.0 mg/dl (⩾353.6 μmol/l) OR Initiation of renal replacement therapy OR, In patients <18 years, decrease in eGFR to <35 ml/min per 1.73 m^2	<0.3 ml/kg/h for ⩾24 hours OR Anuria for ⩾12 hours

Furthermore, we plan daily assessments of renal safety, including daily creatinine, oxalate, and frequent urine oxalate measurements as outlined in detail in the SoA table on page 7. If patients develop AKI, stage 2 or 3 during the first 7 days of the study (4 days of infusion or 3 subsequent days) the following algorithm will be implemented:



If new AKI develops: Plan daily creatinine measurement until improvement in the serum creatinine or urine output occurs for two consecutive days, at which time less frequent assessment as currently proposed in the study protocol can be undertaken.

5.3.3 Patients receiving are receiving therapeutic fluphenazine.
Patients who are taking fluphenazine (e.g., Poloxin) will be excluded.

5.3.4 Special Considerations: Obese patients
Average body weight adults using the VCU protocol for intravenous vitamin C infusion receive 14 – 16 grams of vitamin C infused over a 24-hour period. Because the obese population of patients are known to be at high risk of COVID-19 pneumonia developing into serious respiratory failure we will set a **daily ceiling of 15 grams** infused into patients with BMIs of 40 or above.

5.4 Screen Failures
Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. Effort will be made to collect enough data from screen fails 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).
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 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
Non-intubated patients with COVID19 will be recruited from the Emergency Department (ED), the medicine inpatient wards, and the intensive care units (ICU) at VCU Medical Center and the Central Virginia VA Health System in Richmond, Virginia (RICVA). Both institutions are tertiary care medical centers 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 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.).

6 - Study Intervention

6.1 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 the scheduled time of infusion, a call should be placed to the pharmacy to determine if the study drug may be co-infused into the line that is infusing a different drug. If administering a study drug via co-infusion is contraindicated, then study drug infusion may be delayed by a maximum of 8 hours (the nursing assessments in COVID-19 patients are done every 4 hours). If the clinical drug administration schedule is such that the study drug will not have an available administration time beyond this delay, a dedicated new intravenous line (peripheral) should be inserted. Study drug will be blinded using an identical appearing placebo.
6.1.1 Dosing and Administration

- First study drug dose (L-ascorbic acid, 50 mg/kg infusion over 30 minutes) will be considered "Dose 1" and will be administered within the first 8 hours of enrollment or

the earliest available time post any clinically indicated procedure which requires the patient to be off the unit. Doses will be administered in the ED, medicine inpatient unit or ICU. Patients receiving vitamin C will receive 25% of the total daily calculated dosing 200 mg/kg/24 hours) infused over 30 minutes every 6 hours for 96 hours.

- Subsequent doses will be **50 mg/kg infused over 30 minutes every 6 hours**.
    - Timing of Dose 2 will be triggered by the physician order for every 6-hour administration and will, therefore, be listed on the bedside medical administration record (MAR). As such, the timing of Dose 2 may be out of the +/- 3-hour window and will not trigger a protocol deviation.
    - If for any reason any other maintenance dose is not administered within the window, the dose will be skipped, and the next scheduled dose will be given and documented in the data collection tool.

6.1.2 Concurrent Medication Infused and Renal Acidification

Urine pH will be monitored daily according to the Scheduled of Activities table. Fluoroquinolones, aminoglycosides, macrolides function optimally at alkaline pH. Tetracyclines, nitrofurantoin, many β-lactams exhibit highest activity under acidic conditions. Sulfamethoxazole, oxacillin, amoxicillin and clavulanic acid, vancomycin, imipenem, and clindamycin are unaffected by pH. (32) See section 6.5.2 for a detailed list of all known medication interactions.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

VCU Health and the VA Investigational Drug and/or Inpatient Pharmacy will coordinate the acquisition of sterile L-ascorbic acid (Ascor®) for infusion from McGuff Pharmaceuticals, Santa Ana, CA.

6.2.2 Formulation, Appearance, Packaging, and Labeling

Ascor vials contain 25,000 mg/50 mL of pH-balanced ascorbic acid and is supplied as pharmacy bulk packaging (PBP). The diluted Ascor solution is colorless to pale yellow. The placebo will be 50 ml Dextrose 5% Water (D5%W). **Sealed amber shrouding will be used to cover the IV bag in order to maintain the blind.**

6.2.3 Product Storage and Stability

Ascor is light sensitive. Light exposure will be minimized, and vials will be stored at 2°C to 8°C prior to use. Infusion solutions will be prepared by Investigational Pharmacy (IP) during weekdays and will be placed in the ward refrigerator which is maintained at 2°C to 8°C. IP prepared infusion solutions containing vitamin C or placebo will expire in 24 hours. Infusion solutions prepared by ICU Pharmacy (9^{th} floor Critical Care Hospital) after-hours will expire in 12 hours. There is published data establishing the stability of admixtures of ascorbic acid to be 96 hours stored at room temperature or refrigerated. 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

As noted above VCU Health IP will mix L-ascorbic acid once every 24 hours in four 50 ml hooded infusion bags using aseptic technique according to USP 797 requirements. All Ascor vials utilized will be discarded within 4 hours or sooner as per the Ascor package insert. Prepared solutions are stored on the inpatient unit or ICU refrigerator in the dark at 2°C to 8°C. 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 or loss of activity.

For ascorbic acid, the volume of ascorbic acid to be added to the IV bag will be calculated. **. Under no circumstance an individual infusion dose will exceed 3.75 grams of vitamin C, or the daily infused dose above 15 grams.** This same amount of fluid will be withdrawn out of the D5%W 50 mL bag and discarded. Then, the calculated volume of ascorbic acid will be added to the infusion bag. Residual air will be removed from the infusion bag to prevent oxidation, and the bag will be shrouded. **We commit that all solutions will have a pharmacy-approved appropriate osmolarity for peripheral infusions, and the volume of placebo will be exactly the same as the control.**

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

The investigational pharmacist will use a standardized scientific randomization module within REDCap (36) Only the study pharmacist will have access to the online secure repository. The REDCap module has an auditing trail and log records on who accesses it as well as strict security controls.

The prepared infusion bags will have **sealed** amber shrouding to cover the IV bag in order to maintain the blind.

The randomization method will be a block (per site) and stratified and WHO COVID-19 ordinal outcomes status) randomization.

We plan to perform block randomization for each participating site. Within this block, there will be further stratification of randomization for severity of illness based on WHO COVID-19 ordinal outcome cutoff as seen in the table below:

	WHO COVID-19 scale 3-5 at enrollment	**WHO COVID-19 scale 6-7 at enrollment**
	Stratum A	Stratum B

The goal is for each site to have an equivalent severity among patients randomized to the treatment or placebo arm within each stratum.

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 the standard of care (particularly antibiotics, oxygen therapy, glucose infusion, insulin, albumin, corticosteroids, N-acetylcysteine, norepinephrine, vasopressin) provided will be recorded.

6.5.1 Rescue Medicine

Not applicable.

6.5.2 List of concurrent medications that may be incompatible with intravenous vitamin C

A review of the literature discloses that no known medications are incompatible with intravenous vitamin C concurrent administration. Herein we outline three categories of interacting medications. Medication that are absolutely contraindicated (**none identified**). Medications that may interact in a harmful way, therefore we may need to consider therapy modification. For the study purposes, patients who are taking, or plan to take these medications during the period of the study will be excluded. Medication that will require monitoring. The table below, summarizes the Vitamin C drug interaction groups.

Medication interaction	Interaction Category	Interaction Mechanism	Reference
Aluminum Hydroxide	**Therapy modification Contraindicated for study purposes**	Ascorbic Acid may increase the absorption of Aluminum Hydroxide, especially in patients with renal failure	Domingo JL, Gomez M, Llobet JM, Richart C. Effect of ascorbic acid on gastrointestinal aluminium absorption. Lancet. 1991;338(8780):1467. doi:10.1016/0140-6736(91)92776-x [PubMed 1683458]
Bortezomib	**Therapy modification Contraindicated for study purposes**	Ascorbic Acid may diminish the therapeutic effect of Bortezomib	Perrone G, Hideshima T, Ikeda H, et al, "Ascorbic Acid Inhibits Antitumor Activity of Bortezomib in Vivo," *Leukemia*, 2009, 23:1679-86.
Copper Depends on Dosage Form	**Therapy modification Contraindicated for study purposes**	Copper may decrease the serum concentration of Ascorbic Acid.	Prescribing information. Copper (cupric chloride). Lake Forest, IL: Hospira, Inc., 2004.
Deferoxamine Depends on Dose	**Therapy modification Contraindicated for study purposes**	Ascorbic Acid may enhance the adverse/toxic effect of Deferoxamine. Left ventricular dysfunction is of particular concern.	Prescribing information. Desferal (deferoxamine mesylate). East Hanover, NJ: Novartis Pharmaceuticals Corporation, September 2010.

Amphetamines, fluphenazine	**Therapy modification Contraindicated for study purposes**	Ascorbic Acid may decrease the serum concentration of Amphetamines. Documented discussion with patient, pharmacy and prescribing physician required.	Prescribing information. Adderall (dextroamphetamine and amphetamine). Wayne, PA: Shire US Inc., 3/07. Prescribing information. Dexedrine (dextroamphetamine). Research Triangle Park, NC: GlaxoSmithKline, July 2008.
CycloSPORINE (Systemic)	**Monitoring required**	Ascorbic Acid may decrease the serum concentration of CycloSPORINE (Systemic). **Daily levels are required.** Documented discussion with patient, pharmacy and prescribing physician required.	Lake KD, Aaronson KD, Gorman LE, et al, "Effect of Oral Vitamin E and C Therapy on Calcineurin Inhibitor Levels in Heart Transplant Recipients," J Heart Lung Transplant, 2005, 24(8):990-4. [PubMed 16102431] Prescribing information. Vyvanse (lisdexamfetamine). Wayne, PA: Shire US Inc., June 2013.
Estrogen Derivatives	**Monitoring required**	Ascorbic Acid may increase the serum concentration of Estrogen Derivatives. Documented discussion with patient, pharmacy and prescribing physician required.	Vihtamaki T, Parantainen J, Koivisto AM, et al, "Oral Ascorbic Acid Increases Plasma Oestradiol During Postmenopausal Hormone Replacement Therapy," Maturitas, 2002, 42:129-35. [PubMed 12065172]
Warfarin	**Monitoring required**	More frequent anticoagulation monitoring (**daily INR checks)** may be necessary upon initiation of warfarin therapy with concomitant ascorbic acid. Documented discussion with patient, pharmacy and prescribing physician required.	Sattar A, Willman JE, Kolluri R. Possible warfarin resistance due to interaction with ascorbic acid: case report and literature review. Am J Health Syst Pharm. 2013;70(9):782-786. [PubMed 23592361]
Aspirin	Depending on the dose	Aspirin may decrease the serum concentration of Ascorbic Acid if aspirin is used in the dosing range from 600 mg single dose up to 3.9 g/day. Documented discussion with patient, pharmacy and prescribing physician required.	Basu TK, "Vitamin C-Aspirin Interactions," Int J Vitam Nutr Res Suppl, 1982, 23:83-90. [PubMed 6811490]

Dobutamine, Depends on Route	Depending on the dose	Ascorbic Acid may enhance the positive inotropic effect of Dobutamine. **Monitoring of mean arterial pressure is needed.** Documented discussion with patient, pharmacy and prescribing physician required.	Mak S and Newton GE, "Vitamin C Augments the Inotropic Response to Dobutamine in Humans With Normal Left Ventricular Function," Circulation, 2001, 103(6):826-30. [PubMed 11171790]
Indinavir	Ascorbic Acid may decrease the serum concentration of Indinavir.	Documented discussion with patient, pharmacy and prescribing physician required.	Slain D, Amsden JR, Khakoo RA, Fisher MA, et al, "Effect of High-Dose Vitamin C on the Steady-State Pharmacokinetics of the Protease Inhibitor Indinavir in Healthy Volunteers," Pharmacother, 2005, 25(2):165-70. [PubMed 15767232]
Propranolol	Ascorbic Acid may decrease the serum concentration of Propranolol.	Ascorbic Acid may decrease the serum concentration of Propranolol. Documented discussion with patient, pharmacy and prescribing physician required.	Gonzalez JP, Valdivieso A, Calvo R, et al, "Influence of Vitamin C on the Absorption and First Pass Metabolism of Propranolol," Eur J Clin Pharmacol, 1995, 48(3-4):295-7. [PubMed 7589058]
Reference: Trissel's ™ 2 Clinical Pharmaceutics Database			

6.5.3 List of concurrent medications that should not be admixed, or Y-site administered during IV infusion

No other medication will be Y-site co-administered with intravenous vitamin C without first obtaining approval from hospital pharmacy services. Intravenous vitamin C will be administered through a dedicated IV-line, tubing and pump.

The table below includes medications with known and unknown incompatibility.

Intravenous Medication Y-site administered with IV Ascorbic Acid	Y-Site Incompatibility
Aminophylline	Incompatible
Azathioprine sodium	Incompatible
Ceftazidime	Incompatible
Ceftriaxone sodium	Incompatible
Chloramphenicol sodium succinate	Incompatible
Dantrolene sodium	Incompatible
Diazepam	Incompatible
Diazoxide	Incompatible
Erythromycin lactobionate	Incompatible
Etomidate	Incompatible
Ganciclovir sodium	Incompatible

Hydralazine hydrochloride	Incompatible
Hydroxocobalamin	Incompatible
Inamrinone lactate	Incompatible
Midazolam hydrochloride	Incompatible
Minocycline hydrochloride	Incompatible
Nitroprusside sodium	Incompatible
Papaverine hydrochloride	Incompatible
Pentamidine isethionate	Incompatible
Pentobarbital sodium	Incompatible
Phenytoin sodium	Incompatible
Sulfamethoxazole-trimethoprim	Incompatible
Thiopental sodium	Incompatible
Amphotericin B (conventional)	Unknown
Ampicillin sodium	Unknown
Ampicillin sodium-sulbactam sodium	Unknown
Propofol	Unknown
Haloperidol lactate	Unknown
Reference: Trissel's ™ 2 Clinical Pharmaceutics Database	

6.5.4 Drug or antibiotics interactions that may affected by urine acidification

Intravenous vitamin C may acidify the urine. Fluoroquinolones and macrolides function optimally in alkaline pH and may be affected by acidification of the urine. Fluoroquinolones, aminoglycosides, macrolides function optimally at alkaline pH. Tetracyclines, nitrofurantoin, many β-lactams exhibit highest activity under acidic conditions. Sulfamethoxazole, oxacillin, amoxicillin and clavulanic acid, vancomycin, imipenem, and clindamycin are unaffected by pH. Yang et al. (32).

6.6 Plasma Vitamin C quantification method

Plasma specimens are reduced with dithiothreitol (2mg/ml) followed by precipitation with 20% trichloroacetic acid then vortex-mixed and centrifuged. Supernate vitamin C levels are measured using high-pressure liquid chromatography (HPLC) with UV detection (via cGLP bioanalytical validation according to FDA guidelines on bioanalytical method validation). Chromatography is performed on a Zorbax SB-AQ, 4.6 x 150 mm, 5 µm column (Agilent Technologies, Santa Clara, CA), with a mobile phase using a gradient buffer (dipotassium phosphate), ion pairing reagent (tretrabutyl ammonium chloride), and acetonitrile at a flow rate 0.850 mL/min. Detection will occur at 265 nm and vitamin C levels quantified using peak area analysis and external standardization. Ascorbic acid standards (5 - 2,500 µM) are freshly prepared and treated in the same way as the test plasma samples. The limits of quantification are 5 µM.

7 - Study Intervention Discontinuation and Participant Discontinuation/Withdrawal

7.1 Discontinuation of Study Intervention

There is no defined plan to discontinue the patients from the study, however, the PI may **stop** the vitamin C infusions prematurely in the following conditions, while the other interventions will continue for safety and study reasons:

The study drug will be **stopped** without the PI completely discontinue the study assessments for the patient (i.e. the patient will still participated in blood draw assessments, blood and urine tests per protocol and documentation of outcomes):

- If the patient status improves and patient **downgrades** level of care from ICU to lower levels of care (general or stepdown).
- if participants describe symptoms of **nephrolithiasis** (kidney stones) that is flank pain and hematuria with evidence of kidney stones in the imaging studies.
- do not tolerate the drug due to side effects such as nausea, vomiting, flushing, rash, headache, or diarrhea, the PI will discontinue the study if symptoms are **CTCAE v5 grade 3** or higher and not dose reduce the study drug. In this case the FDA will also be informed.
- if a patient develops a metabolic acidosis **unexplained** by other etiologies (e.g., 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 **stopped** if the primary care team or surrogate decision-maker requests stopping the **drug for any reasons**. Data collection will continue for these patients following the withdrawal of study drug.

7.2 Participant Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request. Upon withdrawal, all study interventions will cease.

7.3 Lost to Follow-Up

Loss to follow-up will be documented in the study registry.

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:

From electronic health record (EHR):

- Demographic information including gender, date of birth, race, ethnicity
- Medical and medication history over the past 30 days
- Documentation of blood for standard of care (SOC) hematology, coagulation, clinical chemistry (if available)

• 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)
• 12-lead electrocardiogram (EKG), Chest imaging findings (Chest X-Ray with infiltrates yes/no; ground-glass opacities on chest CT yes/no) based on the radiological attending report
• Medical Comorbidities and secondary diagnoses
• Use of supplemental oxygen (yes/no)

Study specific

• If not included in SOC, documentation of negative urine pregnancy testing for human chorionic gonadotropin (HCG) if female and of childbearing potential.

Study assessments

The following study data will be documented using standard hospitalization care procedures. Research staff will collect study data from EHR (including process reports) every 24 hours during the study procedure and once a week thereafter.

8.1.1 Venous Blood Collection Timeline

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), while some other centers report undetectable plasma vitamin C in COVID-19 patients**. Baseline ascorbate levels will be drawn, before the imitation of any infusion with subsequent trough levels for PK monitoring.**

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 prior 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).**

Schedule

	Baseline (hour 0)	Treatment days 1-4 (hour 24)	Treatment days 4-7	Days 7-14	Days 14-21	Phase 1 Study end (day 28)	Phase 2 Day 60 and Day 90
Death from ANY cause	X	X	X	X	X	X	X
Alive and Free of any	X	X	X	X	X	X	

respiratory failure							
Alive and free from invasive mechanical ventilation	X	X	X	X	X	X	
AKI (y/n) and stage	X	X	X	X	X	X	
WHO COVD-19 SCALE	X	X	X	X	X	X	X
Concomitant medication use(a)	X	X	X	X	X	X	
Plasma levels (b)	X	X	X	X	X	X	
Vital signs (c)	X	X	X	X	X	X	
Clinical assessment for kidney stone symptoms	X	X	X	X	X	X	
qSOFA score	X	X	X	X	X	X	
Ins/Outs	X	X	X	X	X	X	
Ventilator status / oxygen (e)	X	X	X	X	X	X	
Ventilator-free days	X	X	X	X	X	X	
AKI-free days	X	X	X	X	X	X	
24-hour urine collection			X5, X7	X14*	X21*	X28*	
Ventilator and drip data	X	X	X	X	X	X	
AE/tolerance (g)		X	X				
Mortality (h)	X	X	X	X	X	Date/time	
Discharge (i)	X	X	X	X	X	Date/time	
WHO COVID-19 Scale	X	X	X	X	X	X	

Footnotes:

a. Concomitant medication use (if applicable): oxygen therapy (to calculate S/F ratio), oxygen device, antibiotics, COVID-19-specific (remdesivir, dexamethasone, tocilizumab, sarilumab, canakinumab, hydroxychloroquine, azithromycin, convalescent plasma, remdesivir) corticosteroids, albumin, thiamine, norepinephrine, vasopressin, and N-acetylcysteine, antiplatelet and anticoagulants, muscle relaxants, sedatives (propofol, midazolam) and opioid analgesia.
b. Plasma levels: serum oxalate, creatinine, hematology, coagulation, biomarkers (ferritin, C - reactive protein (CRP), lymphocyte count, LDH, d-dimer). If the primary team does not obtain these tests, we will obtain them for study purposes (dedicated blood draw if needed on day 0 and 7) and as add-on-labs (no study-specific blood draw on days 1-4).
c. Vital signs: Temperature, Respiratory Rate, Systolic and Diastolic Blood Pressures, Heart Rate, RASS score, Alert/ Not Alert, Oriented/ Not Oriented, FiO2, oxygen delivery device.
d. Ins/Outs: 24-hour oral or IV intake/output and dialysis/ CRRT details at midnight.
e. Current ventilation status: use of mechanical ventilator or oxygen device (yes/no), mode, invasive/ non-invasive ventilation, FiO2, PEEP, Plateau Pressure
f. Ventilator/CCRT/Vasopressor/Delirium: last 7 days
g. Adverse Events and tolerance: Interview with patient, may be conducted by telephone if feasible to reduce exposure of study personnel
h. Mortality: date-time of death and all-cause mortality and likely cause of death.
i. Discharge: Discharge date-time and discharge disposition (home, rehabilitation, skilled nursing facility, hospice, transfer to other hospital)
j. For respiratory failure: was the patient at any time: turned to the prone position to facilitate oxygenation, paralyzed, placed on ECMO, had a tracheostomy placed?

Phase 1 End
• Total ventilator free days, CRRT free days, Vasopressor free days, and Delirium free days.
• Is patient still hospitalized?
• For respiratory failure, was the patient at any time: turned to the prone position to facilitate oxygenation, paralyzed, placed on ECMO, had a tracheostomy placed?
• Is patient still alive? If not, date/time and cause of death and code status at time of death.
Phase 2 follow-up (>=90 days)
- Is patient still alive? If not, date/time and cause of death and code status at time of death.

8.2 Safety and Other Assessments
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 table. This trial will use the Common Terminology Criteria for Adverse Events version 5 (CTCAE v. 5) to assess safety and severity of symptoms.

8.2.1 Every 24 hours for as long as the patient is in the hospital: input/output, hypoglycemic events, creatinine, worst vital signs, and oxygen requirement, dialysis, admission to the ICU or need for mechanical ventilation. COVID-19 WHO ordinal status, daily serum chemistries, CBC, magnesium, telemetry ECG tracing monitoring and continuous oximetry monitoring. Patients are monitored for gastrointestinal tolerability (i.e., nausea, diarrhea) and for other adverse events (i.e., rash, headache) and agent will be **stopped** for CTCAE V5 for grade 3 or higher, while the study monitoring will continue.

8.2.2 Every, 24, 48, hours: Acute Kidney Injury-free days, Worst vital signs, Serum electrolytes, chemistries, concurrent medications AE/SAE Assessments, Weight, Bilirubin, Oxygenation Data, Kidney stone symptoms
8.2.3 Every 7, 14, 21 and 28 days: Required: Ventilator Free Days to Day 28, All-Cause Mortality to Day 28, oxygenation indices, oxygen device, use of dialysis. COVID-19 WHO ordinal status.
8.2.4Sixty and 90 Days: All-cause mortality, routine evaluations of organ function, functional status
8.2.5 All patients will be treated **at minimum** subcutaneous unfractionated heparin for thrombo-prophylaxis (5000 units TID). Anticoagulation may be escalated by the treating physicians based on clinical and laboratory variables, such as the D-Dimer and the thromboelastogram (TEG). If suspicion of clotting arises immediate thromboelastography (TEG) will be performed to determine if clotting is ongoing. If TEG supports clotting suspicions doppler ultrasound **of the appropriate venous site** will be performed.
8.2.6 Assessment of hepatic safety: Hepatic transaminases will be monitored at baseline, day 2 and day 3 or the study. Persistent elevations will lead to: 1) discussion with ward staff, 2) will lead to right upper quadrant ultrasonography, and 3) Consultation from the Hepatology service.

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, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or 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, be life-threatening, or require hospitalization 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 SAFE EVICT CORONA-ALI trial will utilize the Common Terminology Criteria for Adverse Events (CTCAE) v5.0, published: November 27, 2017, which grades 1 through 5 with unique descriptions of severity for each adverse event based on the following:

Severity of Event

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental activities of daily living (ADL).
Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care or ADLs.
Grade 4 Life-threatening consequences; urgent intervention indicated.
Grade 5 Death related to AE.

Relationship to Study Intervention

All adverse events AEs will have their relationship to study intervention assessed by the investigator 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.
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 electronic case report form (eCRF). 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 determination), and time of resolution/stabilization of the event. All AEs occurring while on the study must be documented appropriately regardless of relationship. All AEs will be followed to an adequate resolution.
Any medical condition that is present at the time that the participant is screened will be considered as a 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 intermittently 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 involved 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 IRB within 5 business days of receiving notice of the unanticipated problem.
Investigators will report all unanticipated problems, defined as a problem 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 being notified of the event.
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.

CTCAE Grade 3 or higher will trigger a pause in enrollment while the DSMB reviews the data to determine whether subsequent enrollment should proceed.

Examples of untoward clinical occurrences or disease-related events (DRE) that are expected in the course of COVID-19 include: 1) transient or worsening hypoxemia, 2) agitation, 3) delirium, 4) nosocomial infections, 5) skin breakdown, 6) acute hepatitis, 7) **acute kidney injury**, and 8) worsening of respiratory function. 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 individual patient sepsis and AH. 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 Adverse Events, Serious Adverse Events, and Unanticipated Problems

Adverse events (AEs), serious adverse events (SAEs), and unanticipated problems (UPs) occurring at VCU will be managed and reported in accordance with VCU/VCUHS Clinical Research Standard Operating Procedures #CR-RE-300.1: Adverse Event Management and Reporting. Those occurring at the VA will be managed and reported in accordance with the VA's standard operating procedures.

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

The PI/sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after being informed about the event. In addition, the PI/sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potentially serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the PI/sponsor determines that the information qualifies for reporting.

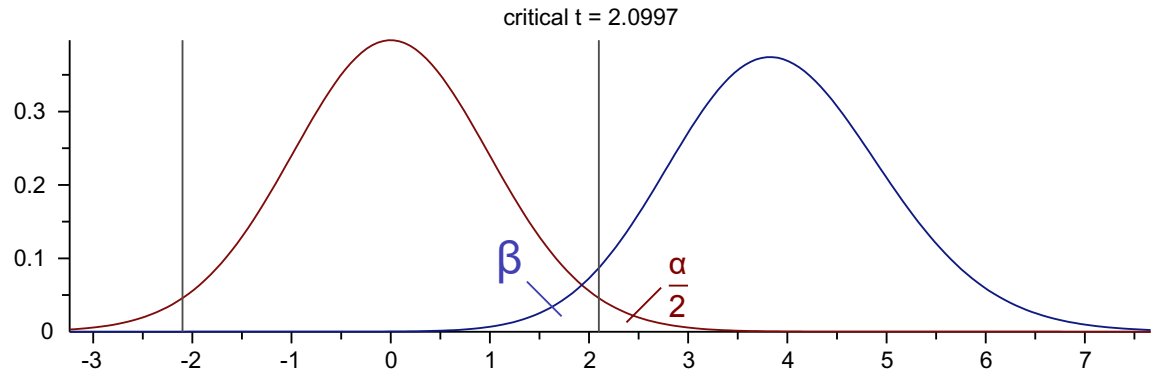
9 - Statistical Considerations

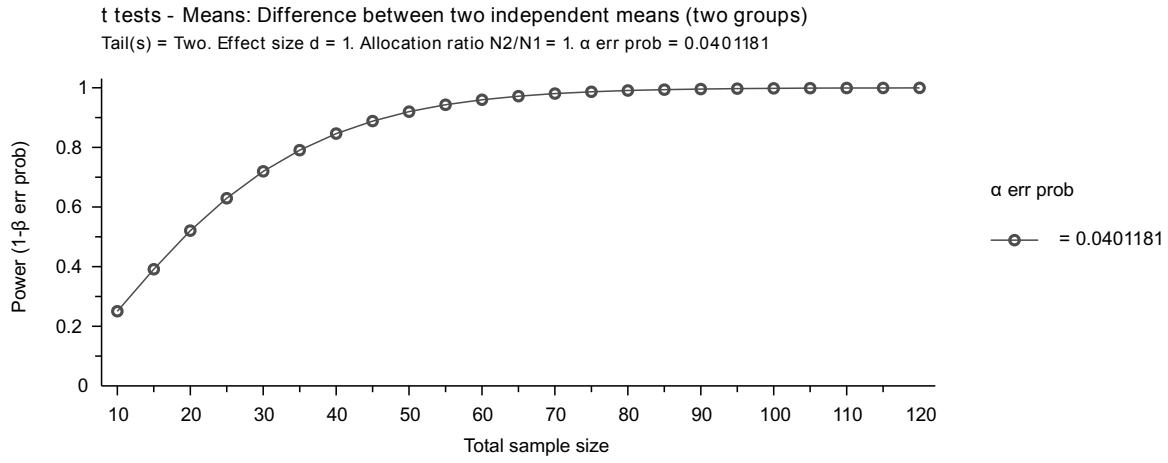
Model Set-Up:

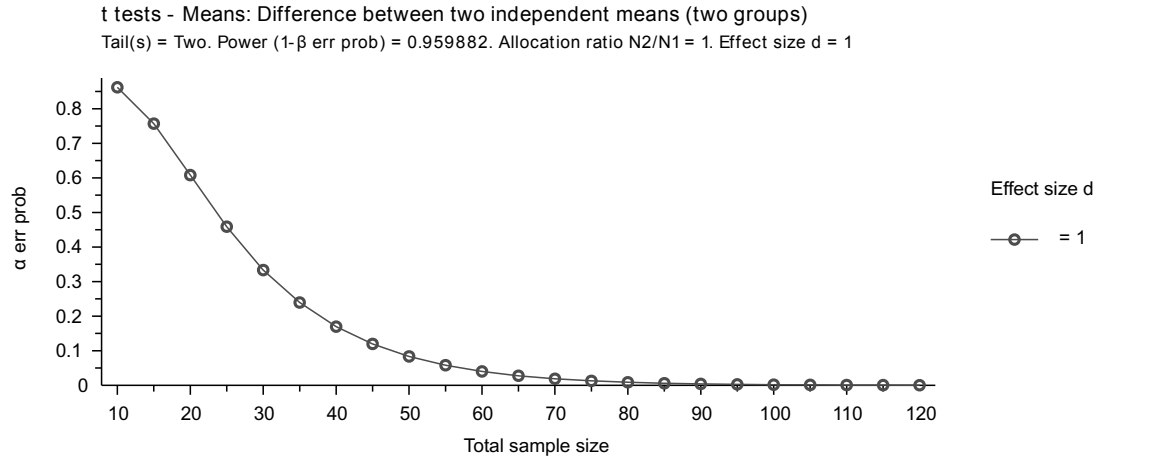
We plan to use a two-sample, unequal-variance 2-tail t-test for comparing the mean differences between the two groups, the vitamin C arm and placebo arm. The actual interventions will be blinded and will be revealed after the completion of statistical analysis, therefore, we will be labeled as group A and group B. No one besides the DSMB and independent statistician supporting the DSMB will have access to the comparative data, even if the treatment groups are masked as group A and Group B.

This is a proof-of-concept study, **with predetermined sample sizes** for each arm, which is 30 patients for group A and 30 patients for group B, therefore the **power analysis is driven by the predetermined sample size and did not determine the sample size.**

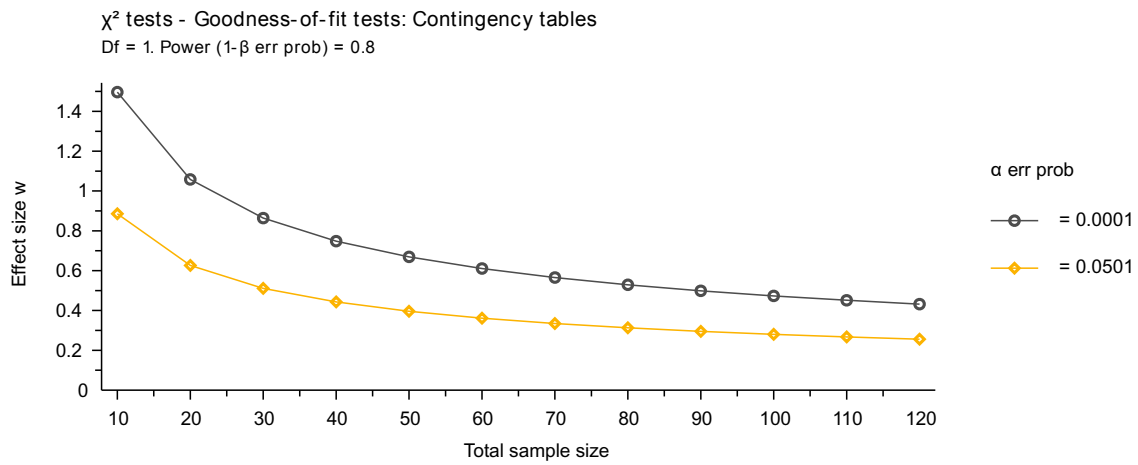
Therefore, this proof-of-concept study of 60 patients, 30 allocated to the treatment arm and 30 to the placebo arm, will have 96% power (1-beta (β)) to detect an **average of 1-point difference** in the primary outcome, the WHO COVID-19 ordinal scale, with an alpha error probability (α) of 4% (0.04). This is by applying a two-tail, t-test with unequal variances (see figure below).

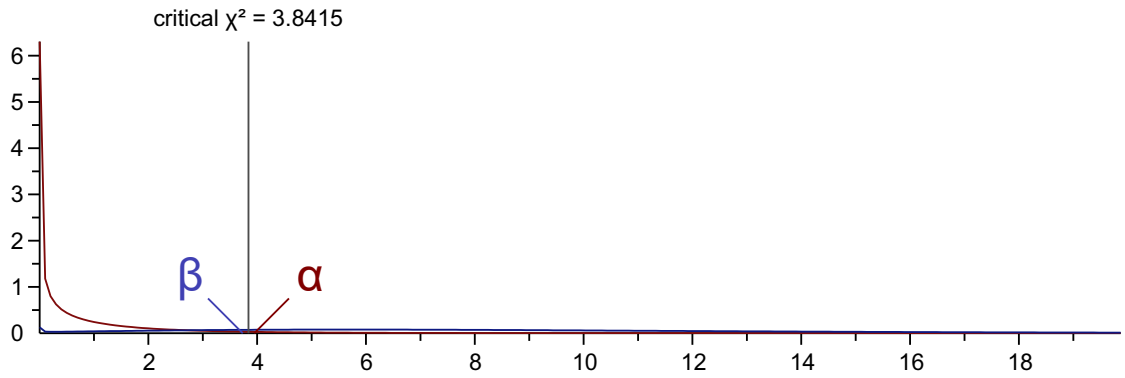




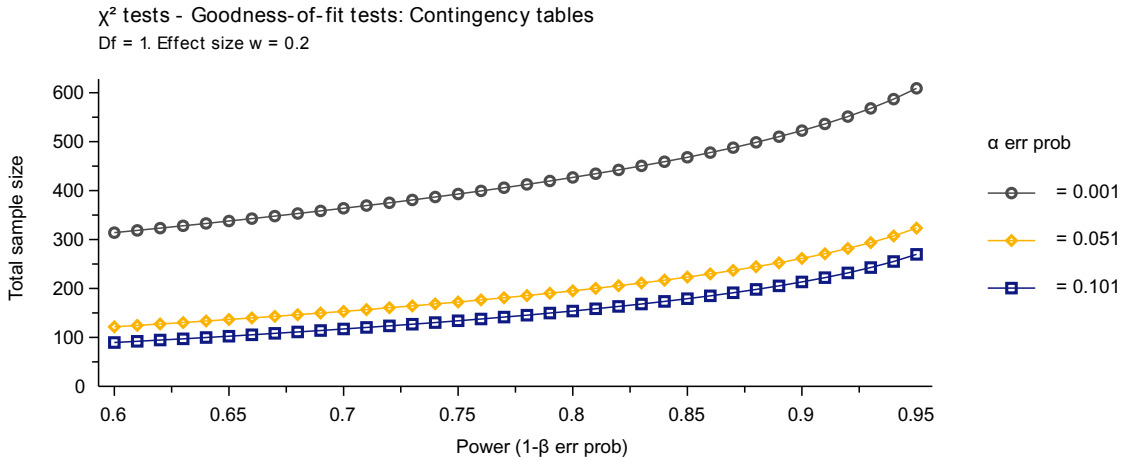


For binary secondary outcomes, we plan to use the chi-squared test, with 1-degree of freedom. The expected effect size for an alpha level of 0.05 and power of 80% is 0.36, as shown in the figure below.





In contrast, for a constant hypothetical effect size of 0.2, a phase 3 trial will require at least 200 patients to achieve a power of 80% and an alpha of 0.05 (yellow line, figure below).



Planned Interim Analyses:

No planned interim analyses will be conducted in this study. The results will be analyzed after the 60th patient has finished enrollment and has been discharged from the hospital (phase 1). There are no provisions to stop the trial early for efficacy.

Missing Data

The primary outcome, the WHO-COVID-19 scale is encompassing missing data from death or hospital discharge. Missing laboratory or clinical data due to patient death, or discharge will not be imputed given this practice will introduce systematic bias, since they are not missing at-random. For organ-system data, the highest scores will be attributed after patient death, since death causes cessation of all organ function, and any imputation method will underestimate the severity. For missing-at-random data, multiple imputation with a pattern-mixture model with control-based pattern imputation will used, with 50 imputed data sets, in order to compensate for data that may be missing at random.

10 - Supporting Documentation and Operational Considerations

10.1 Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Informed Consent Process

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant. Due to the infectious nature of COVID-19, we will follow the FDA guidance dated March 2020 and updated May 12, 2020, to obtain informed consent. In order to protect members of the research team the consent process will be conducted verbally using the existing the same remote system currently being used in the hospital for communication with patients regarding their clinical care. If the participant agrees to participate in the study, their consent will be documented either using an electronic signature, a wet signature which is photographed, attestation by an impartial witness and the investigator that the participant consented, or sterilization of the paper consent. Informed consent will be documented in the EHR prior to starting intervention/administering study intervention.

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 consenting. However, given the time sensitive nature, participants will be given a maximum of 4 hours to make a decision. 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 provide informed consent prior to any procedures being done specifically for the study. Participants will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. Participants will be informed of other COVID-19 trials that are being performed. Should another drug become available to treat COVID-19 that is safe and effective the drug to subjects enrolled in this trial will be offered. 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 consent provided 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.

Some of the patients approached for participation in this research protocol will have limitations of decision-making abilities due to their critical illness. Hence, some patients will not be able to provide informed consent. Accordingly, informed consent will be sought from the potential subject's legally authorized representative (LAR) in compliance with Federal Regulations 45 CFR part 46. Only those individuals who are authorized to serve as LARs per VCU IRB Written Policies and Procedures #XI-3 will be eligible to provide consent on behavior of patients to participate in this study. Because all participants are inpatients in the hospital, the study will use the same LARs for research consent as are acting as LARs for the purposes of treatment.

10.1.1.b Informed Consent with DocuSign Part 11
In concordance with the latest FDA guidance on consent purposes, the study is offering electronic Informed Consent. This will be offered through DocuSign Part 11. The consent forms will be identical in Spanish and English; however, the signatures will be electronic. The electronic informed consent (eIC) will **only be offered** to Legally Authorized Representatives (LAR) **and not in face-to-face consent with patients. For face-to-face encounters only the paper consent forms will be used.**

The consents will be offered in order to facilitate the following:

- Legally Authorized Representative (LAR) may not be able to travel on site in a timely manner.
- LAR may not be comfortable visiting the hospital
- LAR may not be adherent to social distancing/ face masks which make it impossible for study personnel to interact.

The following elements will be followed for the informed consent process, in concordance with the VCU IRB requirements:

a) A remote discussion is required prior to sending the DocuSign Part 11 consent
b) The consent will be emailed or faxed for the LAR to review **prior** to the discussion
c) DocuSign consent cannot be used for in-person interactions. Only the paper ICF.
d) A remote consent discussion is held via phone, Zoom, etc. and the LAR's questions are answered.
e) If the person indicates they are willing, the study team member collects the person's name and email address.
f) The study team member goes into their Part 11 DocuSign account, creates an envelope, and sends it to the participant. An access code will also be set and communicated to the LAR.
g) The LAR clicks the DocuSign email and creates an account password to log in.
h) They review and sign the consent document electronically.
i) The DocuSign envelope is routed to the person obtaining consent for signature and then to the Principal Investigator.
j) Once all signatures are obtained, all persons receive an email and can download a copy of the signed form.
k) The study team (PI, regulatory contact) ensure that the DocuSign Part 11 consent is accessible any time an auditing is requested and a physical copy is held in the study's regulatory binder in a dedicated section of DocuSign Part 11 Informed Consents.

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 (primary outcome, or AKI-free days), or any safety concerns

• Insufficient compliance to protocol requirements
• Data that are not sufficiently complete and/or evaluable
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 Data Confidentiality and Participant Policy

Participant confidentiality and privacy is strictly held in trust by the participating investigators and their staff. This confidentiality is extended to cover testing of biological samples 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 PI.
All research activities will be conducted in as private a setting as possible.
Representatives of the Institutional Review Board (IRB) and regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.
The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or FDA requirements.
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 Human Specimens and Data

Specimens will be added to the Virginia Commonwealth University Registry of SARS-CoV-2 (VCU-RS). The consent process will include the use of completely deidentified and tabulated data for post-hoc studies.

10.1.5 Safety Oversight

Safety oversight will be monitored by the DSMB and co-PI (s) to ensure accurate recording of the data and reporting of any clinically significant AEs to the IRB or appropriate federal authorities.
The Data and Safety Monitoring Board (DSMB) will be comprised of physicians, ethicists, and statisticians from several universities outside of VCU. No one besides the DSMB and independent statistician supporting the DSMB will have access to the comparative data, even if the treatment groups are masked as group A and Group B. The DSMB charter will be submitted for FDA review. The DSMB will have access to masked data to evaluate the benefit-risk of patients enrolled in the SAFE-EVICT-CORONA-ALI trial. The DSMB charter will be submitted for FDA review, specifying the DSMB members. We will include detailed procedures to maintain trial integrity (e.g., personnel, firewalls). We will additionally give the expected enrollment rate and the amount of time to clean and analyze interim data for DSMB review. With the rate of new COVID-19 patient admission we will propose to hold a DSMB review **every month**. The DSMB charter will lay out the steps to ensure review of early interim data which will ensure patient safety.

10.1.6 Key Roles and Study Governance

Not relevant

10.1.7 Clinical Monitoring

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

- 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 a random review of certain data to assess for data accuracy, protocol compliance, and deviations.

10.1.8 Quality Assurance and Quality Control

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution. As mentioned in the statistical section 9.5, for missing at random data, we will employ imputation methods that may include multiple imputations, or pattern mixture models, depending on the appropriateness of each scenario.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

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

10.1.9 Data Handling and Record Keeping

A screening log will be created and stored in a password-protected document on a secure REDCap (18) network. Data will be entered into a password-protected software. Entry logs and audit trails are kept within the system, as well as complete tracing on who entered/ modified what and when. Difference levels of access provided.

The study team will enter the data in REDCap after consent is obtained (31). The PI and co-PIs will supervise the accuracy and integrity of the data.

Study documents should be retained for a minimum of 2 years after study completion.

10.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. 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.

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.

10.1.11 Publication and Data Sharing Policy

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

Data will be shared through the VCU registry for protocols approved by VCU's IRB.

Data may be shared with a national COVID-19 registry.

10.1.12 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.

10.3 Protocol Amendment History

Version	Date	Description of Change	Brief Rationale
1.51	08/14	Change of protocol after FDA PIND feedback	Focusing on a phase 2, safety proof-of-concept study
1.6	9/10	Feedback from FDA (RI)	Revised inclusion and exclusion criteria for AKI, CKD, dosage ceiling of 15 gr daily added. Oxalate measurement as secondary outcome changed to day 5. Dose reduction of 50% for patients with AKI stage 2 or 3.
1.7	9/20	Feedback from FDA Addition of echocardiogram as an exploratory outcome in COVID-19 sepsis patients	Evaluating the effect of vitamin c in the myocardial function and GLS. Correction of errors. Ascorbic acid measurement, adding outcome of alive-and-free of respiratory failure, and alive-and-free of ventilation. Added daily assessments and labs to day 7. Consideration to change the primary outcome to mortality adjusted for prespecified outcomes.
1.8	11/5	Removal of echocardiogram Removal of cf-DNA from secondary outcomes Removal of Syndecan-1 from secondary outcomes Removal of troponin	Budget restriction
1.82	2/7/2021	Due to hospital staffing in the middle of the COVID-19 pandemic we were informed by the VCU Health Clinical Research Team that the study can only be undertaken in ICU level of care. Therefore, we include in the exclusion criteria “non-ICU patients”	Reported Staff Restrictions
1.83	5/22/2021	Clarifications requested by the IRB on inconsistencies when infusions are stopped, status, and clarify discontinuation vs. withdrawal.	After revision of protocol 1.82 this was affected

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