Prospective, Randomized, Double-Blind, Placebo-Controlled Phase II Trial of Intravenous L-Citrulline to Delay and Potentially Prevent the Need for Invasive Mechanical Ventilation for Acute Hypoxemic Respiratory Failure in Patients with COVID-19 (SARS-CoV-2) Illness

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These signatures constitute approval of this protocol and an assurance that this study will be conducted according to all requirements of this protocol.

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1.0 ABBREVIATIONS

ALI	Acute Lung Injury
ARDS	Acute Respiratory Distress Syndrome
CPS1	Carbamoyl-phosphate synthetase 1
AL	Arginine-succinate Lyase
AS	Arginine-succinate Synthase
BMT	Bone Marrow Transplant
CFR	Code of Federal Regulations
CNS	Central Nervous System
CRF	Case Report Form
DC	Discharge
DIC	Disseminated Intravascular Coagulopathy
EBC	Exhaled Breath Condensate
ELISA	Enzyme-linked Immunosorbent Assay
eNOS	Endothelial Nitric Oxide Synthase
FiO_2	Fraction of Inspired Oxygen
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	Informed Consent Form
ICU	Intensive Care Unit
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-8	Interleukin-8
iNOS	Inducible Nitric Oxide Synthase
IRB	Institutional Review Board
IV	Intravenous
L-NMMA	NG-Monomethyl-L-Arginine
MAP	Mean arterial blood pressure
mL	Milliliter
mm Hg	Millimeter of Mercury
NAC	N-Acetyl Cysteine
nNOS	Neuronal Nitric Oxide Synthase
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
NOx	Nitric Oxide Metabolites
O_2^-	Superoxide
OTC	Ornithine Transcarbamylase
ROS	Reactive Oxygen Species
SAE	Serious Adverse Event
S/F	SaO2 / FiO2 ratio
SOFA	Sepsis-related Organ Failure Assessment
TNF	Tumor Necrosis Factor
TNF-α	Tumor Necrosis Factor-alpha
VDI	Vasopressor Dependency Index

2.0 STUDY SUMMARY

Title: Prospective, Randomized, Double-Blind, Placebo-Controlled Phase II Trial of Intravenous L-Citrulline to Delay and Potentially Prevent the Need for Invasive Mechanical Ventilation for Acute Hypoxemic Respiratory Failure in Patients with COVID-19 (SARS-CoV2) Illness.

Objective: To evaluate safety and efficacy of a bolus loading dose and continuous intravenous infusion of L-Citrulline compared to placebo in patients hospitalized with COVID-19 infection (SARS-CoV-2).

Hypothesis: Intravenous L-citrulline administration will safely restore the homeostasis of nitric oxide synthase by increasing both plasma citrulline and arginine levels. We also reason that restoration of citrulline/arginine balance through citrulline administration will safely re-establish homeostasis of NOS, lower oxidative stress, and reduce inflammation, thereby delaying and potentially preventing the need for invasive mechanical ventilation in patients hospitalized with COVID-19 infection (SARS-CoV-2).

Study Design:	Prospective, randomized, placebo-controlled, double-blind design. 1. Enrollment: approximately 6-9 months 2. Plasma citrulline and arginine levels at day 4 will represent the primary biochemical variable. 3. Vasomotor stability, as measured using a vasopressor dependency index (VDI), through study day 10, will represent the primary safety variable. 4. The length of time from the initiation of the treatment to an intubation event for invasive mechanical ventilation measured in hours will represent the primary clinical outcome. 5. Patients will be followed until hospital discharge for mortality, ICU and hospital lengths of stay, and a requirement for invasive mechanical ventilation and non-invasive mechanical ventilation with high flow nasal cannula or BiPAP as additional clinical endpoints. A further comparison will be performed at D28 and D60 based on telephone follow up for those discharged earlier. 6. Patients will exit the study early (prior to 10 days) if they recover and no longer require oxygen therapy.
Treatment Arms:	1. Patients will be randomized to IV Citrulline or placebo 2. Patients will receive an initial bolus of 20 mg/kg (maximum 1500 mg), followed by study infusion of 9 mg/kg per hour (maximum 700mg) for up to 10 days. 3. Patients, healthcare providers, and the sponsor will be blinded to which treatment the patient is receiving. The investigational pharmacy independent pharmacist will be unblinded to the treatment assignment and will deliver the correct infusion to the patient's bedside. 4. The time of initiation of the study drug infusion (either L-Citrulline or placebo will represent time zero. 5. **Intravenous Citrulline:** Patients randomized the citrulline arm will receive an initial loading bolus of 20 mg/kg (maximum 1500 mg) followed by an infusion of 9 mg/kg per hour (maximum 700 mg) for

	a maximum of 10 days. The citrulline solution will be prepared as a 5% (50 mg/mL) isotonic solution in 5% dextrose water. 6. **Placebo:** Patients randomized to placebo will receive equal volume bolus and study infusion of 5% dextrose water for a maximum of 10 days.
Sample Size/Statistical Considerations:	1. The study will enroll up to 66 patients in a 1:1 ratio. 2. Patients will be analyzed on an intention to treat basis. 3. The primary outcome will be time to intubation event—from the initiation of the treatment until intubation for invasive mechanical ventilation—and will be compared using a log-rank test. 4. Plasma citrulline, arginine, and nitric oxide levels will be measured at 2 and12 hours post-bolus and on days 2, 4, 6, 8, and 10 and compared using Linear mixed model or repeated measures ANOVA analysis. 5. The vasopressor dependency index (VDI) will be measured for hemodynamics. Worst vasopressor dependency index score over the first 6 days will be compared using ANCOVA analysis with the baseline index as the covariate. 6. SaO2/FiO2 or PaO2/FiO2 ratio will be utilized to measure the progression of ALI. Worst values from days 0, 2, 4, or 6, 8, or 10 will be compared between groups using repeated measures ANCOVA with the baseline value as the covariate.
Inclusion Criteria:	1. Age 18-65 years inclusive. 2. Clinical and laboratory evidence of COVID-19 (SARS-CoV2) infection with an acute hypoxemic respiratory illness requiring hospitalization and oxygen therapy. 3. Admitted and transferred to floor without intubation.
Exclusion Criteria:	1. Subject unable to provide consent. 2. Patient, surrogate, or physician not committed to full support. 3. Malignant or other irreversible condition and estimated 28-day mortality ≥ 50% 4. Moribund patient not expected to survive 48 hours (as defined by the primary medical team) from the start of study infusion 5. End-stage Liver Disease as defined by Child-Pugh Score > 9 6. Currently enrolled in, or having participated, in another study of an investigational compound within the last 30 days 7. Pregnant female or female who is breastfeeding 8. Allergy to L-citrulline or arginine or any citrulline- or arginine-containing product 9. Patient not otherwise suitable for the study in the opinion of any of the investigators 10. Requirement for intubation and invasive mechanical ventilation prior to study enrollment

Primary Clinical Efficacy Variables:	The primary clinical efficacy variable is time to intubation event in hours from the start of study infusion. Primary efficacy variables will be evaluated comparing intravenous L-citrulline vs. placebo in separate analyses.
Primary Biochemical Efficacy Variables:	The primary biochemical efficacy variable is plasma levels of citrulline that are associated with primary and secondary clinical efficacy outcomes. It may serve as a surrogate marker for response.
Primary Safety Variable:	Primary safety variable is hemodynamic status, specifically worst vasopressor dependency index through day 10.
Secondary Clinical Efficacy Variables:	1. All-cause hospital mortality 2. The requirement for intubation and invasive mechanical ventilation 3. Length of non-invasive mechanical ventilation in hours 4. Length of oxygen therapy in hours 5. Length of ICU stay in hours 6. Length of hospital stay in hours 7. Difference in proportion of patients intubated 8. Difference in proportion of patients intubated and on MV 9. Duration of mechanical ventilation from consent & post-infusion
Secondary Biochemical Efficacy Variables:	1. Plasma levels of citrulline and arginine at 2 hours, 12 hours, and study days 2, 4, 6, 8, 10. 2. Plasma NOx levels at 2 hours, 12 hours, and study days 2, 4, 6, 8, 10. 3. PK-PD analysis/modeling will enable dose proportionality (dose-response effect) determinations for a follow on confirmatory trial.
Secondary Safety Variables:	Incidence of reported adverse events to include D28 and D60 in-hospital or telephone follow up
Safety Evaluations:	In addition to vasopressor dependency index, the following laboratory values will be used for safety assessment at baseline and daily through study day 4 when available. 1. Complete blood count with platelets 2. Basic Metabolic profile, including renal function 3. Liver function tests, including SGOT, SGPT, total bilirubin, and alkaline phosphatase Adverse events will be recorded prospectively, including incidences of hypotension, hepatitis (i.e., the elevation of liver enzymes), and injection site reactions.

3.0 INTRODUCTION

3.1 The Urea/NO Cycle

A critical biochemical pathway to maintain metabolic homeostasis is the urea/nitric oxide (NO) cycle [1]. The urea/NO cycle is involved in the intermediary metabolism of the key amino acids citrulline and arginine, forming a critical bridge between urea/NO cycle activities in the liver and the nitric oxide synthase (NOS) system present in other tissues for cell signaling and immune functioning. Alterations in the urea cycle in the liver can lead to systemic effects, including oxidant stress and inflammation (Figure 1). Kupffer cells, the largest fixed macrophage population in the body, have the capacity to react to various inflammatory insults, including infectious agents, and release inflammatory cytokines like TNFα, IL-1, and IL-8, into the circulation [2,3]. In addition, although the full urea cycle is limited to the liver, other organs, including the lung, contain many of the cytosolic enzymes, including nitric oxide synthase (NOS). The degree to which Kupffer cells and other macrophages, including pulmonary macrophages, deal with oxidant stress, and maintain metabolic homeostasis is closely related to NOS function and availability of arginine. NOS, in its natural coupled form, utilizes arginine as a substrate for producing nitric oxide (NO) and citrulline. However, when uncoupled, NOS preferentially produces the superoxide radical (O_2^-). This free radical reacts with NO to form peroxynitrite and other ROS, which stimulate monocytes to produce and release additional proinflammatory cytokines [4,5], further propagating and prolonging the inflammatory cascade (Figure 1).

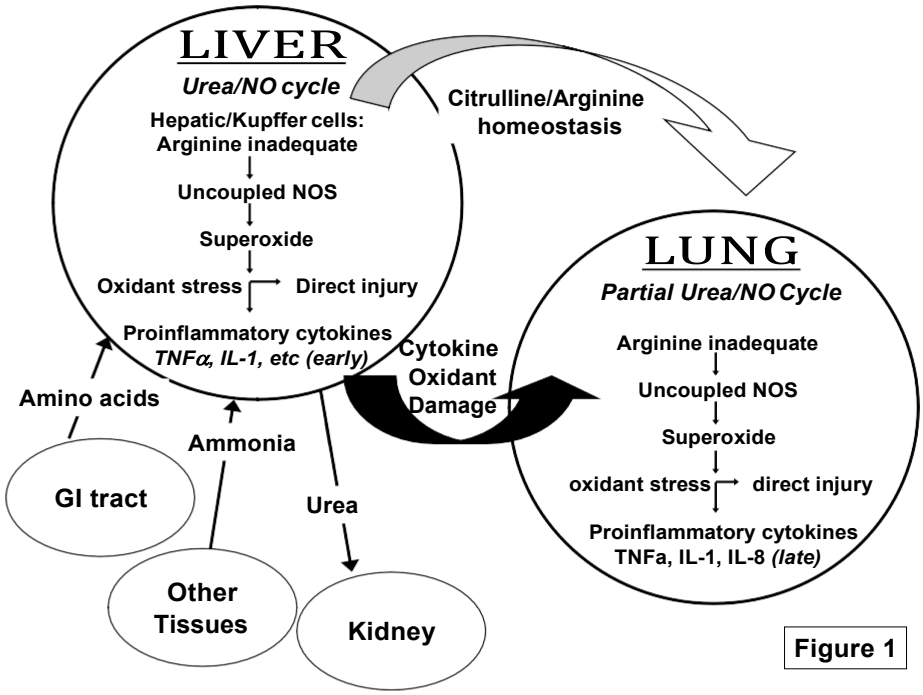


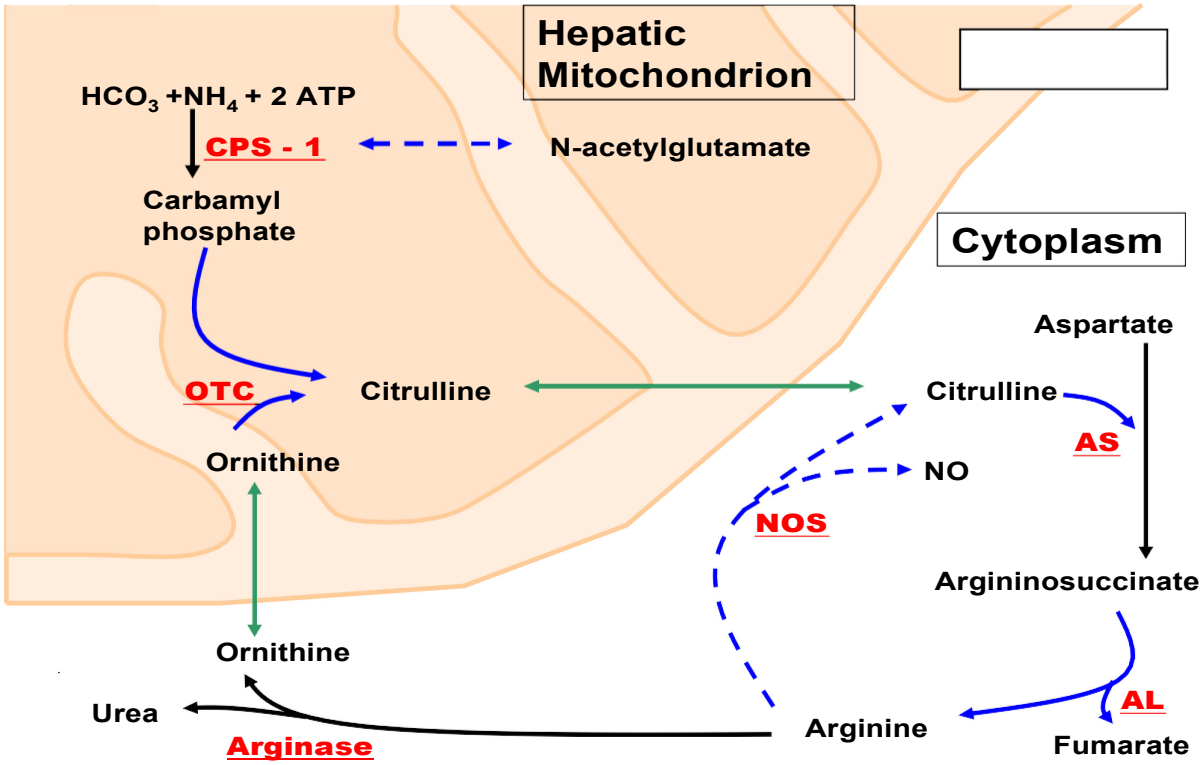
Figure 1

The rate-limiting enzyme catalyzing the first committed step of ureagenesis in the urea/NO cycle, carbamoyl-phosphate synthetase 1 (CPS1) (Figure 2), is highly tissue specific, with function and production limited to the liver and small intestine. CPS 1 forms carbamoyl phosphate from ammonia,

carbon dioxide, and ATP-derived phosphate, which is then joined to ornithine by ornithine transcarbamylase (OTC) to form citrulline. Citrulline is not found in any enzyme or structural protein, thus its principal function is its role in the urea cycle. The vast majority of both CPS1 and OTC are found in hepatic mitochondria. The remaining enzymes, arginine-succinate synthase (AS), arginine-succinate lyase (AL), and arginase are cytoplasmic enzymes with wide tissue distribution, including the lung [6]. Sequential actions of AS and AL convert citrulline to arginine, the only non-dietary source of arginine. The liver either takes up citrulline from GI production/absorption or manufactures it from ornithine and converts it to arginine, releasing the latter into the bloodstream. Ordinarily in the liver, urea is cleaved from arginine to regenerate ornithine. However, arginine exported from the liver also serves as a substrate for systemic NOS, heme proteins that catalyze oxidation of L-arginine to NO and L-citrulline. NOS is found in all cells in at least one of three isoforms: nNOS (neuronal, constitutive), eNOS (endothelial, constitutive), and iNOS (inducible). NOS substrate, arginine, is manufactured in the cell from citrulline or is supplied to the body by the liver or the kidney. Under normal physiological conditions, homeostasis of NOS activity is maintained by levels of both citrulline and arginine.

Within the endothelial cell, the chemically bound caveolar HSP-90 pathway restricts NO production to arginine derived from HSP-90-bound citrulline. In addition, we have recently observed that in human vascular endothelial cells, extracellular citrulline and not arginine, is the effective precursor of NO production. These findings suggest that circulating levels of citrulline may be more predictive of NOS function than arginine levels. Citrulline serves as a negative feedback inhibitor on NOS and arginine, couples NOS to produce NO preferentially over O_2^-.

There are clear links between a poorly functioning urea/NO cycle in the liver, altered balance of citrulline and arginine, decreased NO production in the lung and increased oxidant stress, which results in increased pulmonary vascular resistance, development of ALI and mortality. We also reason that restoration of citrulline/arginine balance through citrulline administration will safely re-establish homeostasis of NOS, lower oxidative stress, and reduce inflammation without resulting in overproduction of NO and precipitating hypotension.



3.2 The Urea/NO Cycle and Oxidative Stress in Acute Hypoxemic Respiratory Failure

Although the biochemical relationships and interlocking metabolic pathways in health and disease are complex, the urea cycle and NO metabolites play an integral role in the inflammatory cascade and oxidative stress reactions of many disease states. Homeostasis of redox signaling is contingent on many aspects which are complex, take place in a variety of tissues and depend on a balance of competing factors, including the amount and location of arginine, citrulline, NO and superoxide generation as well as whether or not these reactions are coupled [7]. As such, citrulline and arginine form a critical bridge between urea/NO cycle activities in the liver and the NOS system for cell signaling and immune functioning in the liver, lung, microvasculature, and elsewhere in the body. Intracellular pools of citrulline primarily and arginine determine NO synthesis and play a major role in oxidative stress. It has been shown *in vitro* that iNOS superoxide generation is decreased substantially with physiologic concentrations of arginine and is abolished completely in similarly designed nNOS systems [8,9].

Inducible NOS is upregulated during physiologic stress; however, if NOS substrate is insufficient then NOS can uncouple and switch from NO generation to production of damaging peroxynitrites. We hypothesized that NOS substrate levels are low in patients with COVID 19 (SARS-CoV2) and that low levels of the NOS substrate citrulline would be associated with end organ damage including acute respiratory syndrome. We further hypothesized that arginine levels would not be associated with acute respiratory syndrome because of substrate channeling from citrulline to NO. Furthermore, citrulline enters the cell with relatively little competition through neutral amino transport, while arginine uses dibasic amino acid transport, and we detect very little free arginine inside cells, findings that also favor a primary role for citrulline availability in regulation of NOS coupling.

In inflammatory conditions, ROS are produced within endothelial cells and neutrophils. These free radicals interact with endothelial cell membranes, especially in the lung, via lipid peroxidation, causing altered membrane fluidity and function. In acute respiratory failure and other inflammatory states, the urea cycle/NO system plays an integral role in this process. Intracellular deficiency of arginine and its precursor citrulline is common due to a combination of poor intake of substrate and reduced activity of CPS1, due to oxidation then proteolytic degradation. In addition, AS and AL are often co-induced along with inducible NOS (iNOS), especially in macrophages, making the citrulline-arginine cycle hyperactive in many inflamed tissues, including the lung in patients with severe infections. The resultant decreased production and increased consumption of citrulline and arginine results in dramatically decreased intracellular and plasma levels of both [10], which have been linked to abnormal liver function, lung injury, coma, and death [11-13]. The hepatic and systemic deficiency of arginine, like those seen in severely septic or BMT patients, contributes to the uncoupling of NOS. Uncoupled NOS preferentially generates O_2^- rather than NO [8,13-15] (Figure 2). Additionally, citrulline becomes a conditionally essential amino acid in these inflammatory states as levels fall below those needed to maintain negative feedback inhibition on NOS, resulting in hyperactive NOS and overproduction of both NO and O_2^-. Increased NO production results in vasomotor instability and contributes to the hypotension seen in septic shock. The subsequent reaction between NO and superoxide generates peroxynitrite [16], which contributes to vasomotor dysfunction [17,18] and may inactivate catecholamines [19]. Peroxynitrite also reacts with unsaturated fatty acids resulting in lipid peroxidation [20]. Lipid peroxidation of the endothelial cell membranes result in altered membrane structure and function which leads to capillary permeability and the syndrome of ALI. In addition, the resultant oxidative stress stimulates monocytes to release more pro-inflammatory cytokines, further potentiating the inflammatory cascade and subsequent end-organ damage, especially lung injury.

3.3 Citrulline Replacement

As mentioned, citrulline's main function appears to be related to the urea/NO cycle, and it may be a better molecule than arginine to support this function for several reasons. First, citrulline has free access to the mitochondrial matrix and facile entry into the urea cycle pathway. Specific dibasic amino acid transporters allow shuttling of ornithine and citrulline across the mitochondrial membrane [21]. Dietary manipulation of citrulline content clearly influences urea cycle intermediate levels. Addition of citrulline to the diet of arginine deficient rats supports a normal level of arginine in the blood, reduces plasma ammonia to normal, and eliminates excretion of orotic acid in the urine [22]. Recycling of citrulline to arginine is well documented in several species and in many tissues [23]. In a study of 30 patients with Reye's syndrome, a disease whose characteristics are similar to sepsis with depression of CPS1 activity and markedly decreased plasma citrulline levels, provision of enteral citrulline (n=8) promoted more rapid lowering of plasma ammonia and among the sickest patients, showed a trend toward reduced mortality compared to untreated patients (n=14) (50% vs. 88%; P=0.075) (DeLong 1982). In healthy adults, patients undergoing chemotherapy for bone marrow transplant, and children undergoing congenital heart surgery, supplementation with citrulline (either oral or IV) supports a higher plasma level of arginine and is associated with decreased *in vivo* lipid peroxidation suggesting that citrulline is superior to arginine as a method of enhancing circulating arginine and reducing oxidative stress.

In elegant studies of plasma arginine, citrulline, ornithine, and NO flux using stable isotopic tracers [24-30], Castillo and colleagues showed that: (1) Arginine-free intake does not significantly affect the rate of either *in vivo* arginine or citrulline biosynthesis, despite a decline in the plasma arginine flux and free arginine concentration. (2) Temporary arginine-free diet did not reduce total nitrate output (i.e. NO production was not reduced). Together, these suggest that a reduction in arginine does not limit citrulline

or NO production (3) Labeled citrulline leaves the plasma and is metabolized by a full turn of the urea cycle before reentering as arginine, (4) Oral administration of ^{15}N-arginine results in a nearly five-fold greater recovery of tracer in $^{15}NO_3$ than when an equimolar amount of tracer is given intravenously, documenting significant splanchnic production of NO. Administration of IV citrulline significantly bypasses the splanchnic circulation, thus limiting the splanchnic production of NO. (5) About 15% of the plasma arginine turnover is associated with urea formation; only 1.2% is associated with NO production. As arginase is upregulated in sepsis, the amount of arginine available as substrate for NOS is further reduced. Although this may decrease NO production somewhat (likely limited due to the low percentage of arginine converted to NO normally), it also alters homeostasis by uncoupling NOS and limiting the amount of NO available to scavenge free radicals. Citrulline is resistant to arginase and our data demonstrate that citrulline administration produces a more consistent plasma level of arginine than arginine administration itself.

3.4 COVID-19 (SARS-CoV2) and Acute Hypoxemic Respiratory Failure Requiring Invasive Mechanical Ventilation

Although reports in the US literature on COVID-19 (SARS-CoV2) and critical illness is limited, a recent case series published in the New England Journal of Medicine detailed the clinical course of 24 critically ill adults with acute hypoxemic respiratory failure and confirmed COVID-19 infection (SARS-CoV2) in Seattle, Washington at 3 hospitals in the University of Washington health care system (106). The mean age was 64 +/- 18 years with a range of 23-97 years and 63% were male. Patients had symptoms an average of 7 days before hospital admission. Of the 24 patients, 18 or 75% required invasive mechanical ventilation but after exclusion of 3 patients who had “do not intubate” directives the percentage was to 18/21 or 86% requiring invasive mechanical ventilation. The median duration of invasive mechanical ventilation was 10 days (IQR 7-12) and overall mortality was 50% in this critically ill population.

3.4 Summary

The body lives in a delicate balance of homeostasis. The urea/NO cycle plays a critical role in maintaining redox homeostasis and as such, also plays a role in regulating inflammation. The biochemical relationships are complex and depend on inter-organ transfer, membrane transport, and intracellular compartmentation. However, data above demonstrate that citrulline, arginine, and NO are critical in maintaining this homeostasis through their regulation of NOS. Inflammation, especially from infection, results in decreased activity of CPS1 and increased activity of arginase, which decreases levels of both citrulline and arginine. These decreased levels result in dysregulated and uncoupled NOS, which drives both overexuberant NO production and formation of ROS. Both the NO production and ROS further exacerbate the inflammatory cascade, resulting in other organ dysfunctions, including acute lung injury. Both inflammation and oxidative stress have been shown to be driving forces for the development of ALI and *regulated* NOS function is vital to reducing both. Both plasma citrulline and arginine are deficient in sepsis and levels are inversely associated with development of ALI. Furthermore, citrulline replacement safely increases plasma levels of both citrulline and arginine in healthy volunteers, BMT patients, adults with sepsis, children with sickle cell disease, and children after congenital heart surgery. It seems highly likely that citrulline therapy in the setting of COVID-19 (SARS-CoV2) induced acute hypoxemic respiratory illness will safely increase citrulline and arginine levels and help re-establish NOS homeostasis, resulting in NO production in compartments that are more homeostatically appropriate so as to reduce pulmonary vascular resistance and enhance coupling of NOS to minimize superoxide production thus reducing free radical mediated ALI.

4.0 OBJECTIVES

4.1 Primary Endpoints (objectives)

The primary objectives of the study are as follows:

- The primary biochemical objective of this trial is to evaluate the effects of intravenous L-Citrulline on plasma levels of citrulline and arginine in patients admitted to the hospital with COVID-19 infection (SARS-CoV2) and acute hypoxemic respiratory symptoms requiring oxygen therapy. An association of plasma amino acid levels to clinical outcomes may serve as surrogate marker for response.

- The primary safety objective is a beneficial effect of intravenous L-Citrulline on hemodynamics.
- The primary clinical objective is to evaluate the difference in the length of time to an intubation event in hours from the start of study infusion between the study arms.

4.2 Secondary Endpoints (objectives)

The secondary objectives of this study are as follows:

- To evaluate the safety of intravenous L-Citrulline compared to placebo as measured by incidence of reported adverse events.
- To evaluate the effect of intravenous L-Citrulline compared to placebo as measured by the total length of all mechanical ventilation, including non-invasive modalities such as high flow nasal cannula and BiPAP and oxygen therapy.
- To evaluate the effect of intravenous L-Citrulline compared to placebo on Hospital all-cause mortality
- To evaluate the effect of intravenous L-Citrulline compared to placebo on lengths of ICU and hospital stay
- To evaluate overall difference in intubation rates
- To evaluate overall duration of mechanical ventilation from consent and post-infusion

5.0 STUDY DESIGN

5.1 Study Description

This is a randomized, double-blind, placebo-controlled, phase II study to evaluate the safety and efficacy of intravenous L-Citrulline in reducing the length of time from the start of study infusion to an intubation event in hospitalized acutely ill patients with an acute hypoxemic respiratory illness due to COVID-19 infection (SARS-CoV2). Patient's age of 18 years old and less than 65 years old inclusive with COVID-19 infection (SARS-CoV2) will be included in the study.

Only subjects hospitalized at UAMS and requiring oxygen for an acute hypoxemic respiratory illness due to COVID-19 (SARS-CoV2) will be included in this study. Study drug administration will occur exclusively in the hospital.

In addition, subjects will receive standard supportive care for acute hypoxemic respiratory illness and failure.

Up to 66 participants ages 18 years to 65 years, inclusive, who meet all the inclusion criteria and none of the exclusion criteria will be randomized into the study over approximately 6-9 months.

The study consists of an initial Study Drug bolus followed by a continuous infusion for a maximum of 10 days. The safety and efficacy will be evaluated through hemodynamic monitoring, laboratory assessment, oxygenation status, and biomarker measurements through study day 10. Adverse events will be monitored through study day 10. Clinical outcomes, including all-cause mortality, ICU length of stay, and hospital length of stay will be assessed through hospital discharge with a telephone follow up at D28 and D60, if discharged earlier

5.1.1 Randomization, Stratification, and Blinding

Participants will be randomly assigned to one of two study groups. Randomization assignments will be determined prior to initiation of the study using a random size permuted block design. Randomization will be stratified by age group with participants divided into 2 age groups of 18-49 years and 50-65 years. After obtaining informed consent, the signed informed consent document will be faxed to the investigational pharmacy. Upon receipt, the investigational pharmacy will begin preparation of the study material infusion. Assignment will be communicated electronically using Medrio randomization system. Once the study drug material is prepared, it will be sent to the bedside for administration via the bedside nurse under the oversight of one of the site investigators and/or study coordinator. After baseline hemodynamic assessments, participants will be randomized to either placebo or citrulline in a 1:1 fashion stratified by age group. The participants, nurses, physicians, study coordinators and investigators will remain blinded to whether participants are receiving placebo or L-citrulline throughout the study. The investigational pharmacy will be unblinded and will be responsible for maintaining the blinding of investigators, participants, nurses, and primary medical team. The investigational pharmacy will deliver the correct infusion(s) to the patient's bedside every 24 hours. The placebo and study drug solutions will be identical appearing on delivery to the ICU where the drug will be administered.

5.1.2 Study Assessments

The assessment of efficacy will include collection of plasma for analysis of citrulline and arginine levels at day 1 hours 0, 2, 12, and the alternating morning of study days the infusion is running for a maximum of 10 days, i.e., on days 2, 4, 6, 8 and 10. A patient will exit the study early if they have been weaned off all oxygen support before 10 days. Additional efficacy measures will include clinical outcomes, including progression of acute lung injury and lengths of hospital and ICU stay.

The assessment of safety will include collection of hemodynamic measurements at least every 4 hours on a hospital unit and--if a patient is critically ill in an ICU--hourly while the study material is infusing and for 12 hours after completion of the infusion in each patient. Hemodynamic measurements will be converted to a vasopressor dependency index for analysis if a patient becomes critically ill. Adverse event reporting will also serve as a means of safety analysis. Additional safety measures will be biochemical and hematologic laboratory measurements. The biochemical laboratory measurements, including liver function tests (ALT, AST, bilirubin, alkaline phosphatase), kidney function tests, and electrolytes, along with hematologic laboratory measurements, including complete blood count and platelet count, will be collected when available for any study day in order to evaluate for adverse events.

5.2 Treatment Arms and Study Drug Administration

All study material infusions (including the initial boluses) will be administered intravenously through a dedicated peripheral line or a dedicated port of a central line using a volumetric or syringe pump. The infusion will be restarted as soon as possible after any stoppages and will continue through day 10 unless the patient exits the study early. Regardless of amount of time the infusion is stopped, time missed during any stoppage will not be added to the end of the infusion.

5.2.1 Citrulline Arm

Patients randomized to citrulline will receive an initial intravenous bolus of 20 mg/kg (to a maximum of 1500 mg) L-citrulline over 10 minutes. The study solution will be prepared as a 5% isotonic solution (50 mg/mL) in 5% dextrose water. Immediately after the initial bolus, a continuous intravenous infusion of L-citrulline at 9 mg/kg (max 700 mg) per hour will be administered through a dedicated intravenous line or port of a multi-lumen catheter.

5.2.2 Placebo Arm

Patients randomized to placebo arm will receive an infusion of 5% dextrose water matched for volume and color to the citrulline infusion. The placebo infusion will consist of an initial iv bolus (up to 30 mL) over 10 minutes followed by a continuous infusion of 5% dextrose water (about 15 mL/hr). The initial bolus and subsequent infusion will be administered through a dedicated intravenous line or port of a multi-lumen catheter.

5.2.3 Drug Interruptions

Interruptions of study infusions should be avoided. As the study drug poses no known risk during procedures, infusions should be continued during bedside procedures. If the study infusion must be interrupted, it should be restarted at the same infusion rate as soon as possible. In cases where the infusion was interrupted, the infusion window will be restarted as soon as possible but will not be extended beyond the end of study day 10. Any study infusion interruptions will be recorded, including the reason for the interruption.

In the case of a significant safety concern related to the drug administration, the study drug should be discontinued until the situation is evaluated by the safety officer. Since there is no specific antidote for L-citrulline, in almost all cases simply discontinuing the study drug is appropriate. Breaking the blind will not generally provide increased safety. The study medication blind shall not be broken unless information concerning the study medication is clearly necessary for the medical treatment of the participant. The decision to unblind study personnel for an adverse event or other circumstance will be determined by the safety officer in consultation with the investigators. If unblinding occurs, the investigator will notify the IRB within 24 hours. The date, time, and reason that the blind was broken will be provided and documented in the CRF.

6.0 STUDY POPULATION AND ENROLLMENT

6.1 Participant Enrollment

Up to 66 participants will be enrolled. The study is expected to accrue over about 6-9 months. Participants will be recruited from the University of Arkansas for Medical Sciences Hospital. Additional

sites may be added at the discretion of the investigators and the sponsor. Participants who withdraw or prematurely discontinue study material infusion will be included in an intention to treat analysis. Safety data will continue to be collected up to hospital discharge for any participants who are withdrawn from the study.

6.2 Inclusion Criteria

Participant eligibility is determined based on the selection of criteria detailed below:

1. Age 18-65 years.
2. Clinical evidence of COVID-19 (SARS-CoV2) infection, defined as a positive COVID-19 laboratory test plus evidence of an acute hypoxemic respiratory illness requiring oxygen.
3. Admitted and transferred to floor without intubation.

6.3 Exclusion Criteria:

Patients meeting any of the following criteria will not be eligible for participation in the study:

1. No consent/inability to obtain consent
2. Patient, surrogate, or physician not committed to full support
3. Malignant or other irreversible condition and estimated 28-day mortality $\geq 50\%$
4. Moribund patient not expected to survive 48 hours (as defined by primary medical team) from start of study infusion
5. End-stage Liver Disease as defined by Child-Pugh Score > 9
6. Currently enrolled in, or participated in another study of an investigational compound within the last 30 days
7. Pregnant female, or female who is breast feeding
8. Allergy to L-citrulline or arginine or any citrulline- or arginine-containing product
9. Patient not otherwise suitable for the study in the opinion of any of the investigators
10. Requirement for intubation and invasive mechanical ventilation before study enrollment

6.4 Informed Consent

Study personnel (either coordinator or investigators) will identify potential candidates for enrollment utilizing the inclusion and exclusion criteria per protocol. Once a potential participant is identified, the study coordinator or principal investigator will meet with the patient and/or legally authorized representative and will describe the proposed study protocol in lay terminology. Prior to performing any study procedures, an IRB-approved informed consent form (ICF) must be signed and dated by each study participant or legally authorized surrogate representative and the study investigator or person informing the subject and obtaining the consent. Prior to taking part in the study, the participant or his or her legal representative should receive a copy of the signed and dated ICF. This procedure will be noted in the medical record. ICF will include PK sample retention for any exploratory analysis of certain intermediates of special interest to support further understanding of the role of citrulline.

A special vulnerable population, specifically cognitively impaired adults, will be eligible for enrollment in this proposed study. Although not all enrolled participants will be cognitively impaired, many could be. In cases where the potential participant is cognitively impaired, surrogate consent will be obtained from the participant's legally authorized representative according to state law. In these cases, the

participant will be "re-consented" at the earliest opportunity and given the option of continuing or stopping their participation.

All revised informed consent forms must be reviewed in the same manner and signed by any active participants or their legal representatives. The date that the informed consent is obtained will be documented in both the source documents and the case report form (CRF).

A subject number will be assigned to each participant after the ICF is signed. Numbers will be unique 3-digit numbers, assigned in ascending order starting with 001.

6.5 Excluded Medications

As interactions between citrulline and other medications are not known, no specific medications will be excluded. Patients should not receive exogenous citrulline or arginine (either intravenously or enterally) while participating in this study. Additionally, patients enrolled in another study of an investigational medicine will be excluded from participating.

6.6 Concomitant Medications

All medications administered as part of standard supportive care for acute hypoxemic respiratory failure will be allowed, including antibiotics, replacement dosages of corticosteroids, catecholamines and other vasopressors, and insulin for glucose control.

6.7 Criteria for Discontinuation or Withdrawal

The primary reason for treatment discontinuation will be noted in the case report form using the following categories:

a. Adverse Event: The participant has experienced an adverse event that the investigator believes requires early termination because continued participation imposes an unnecessary risk to the participant's health.

b. Major Protocol Deviation: the enrolled participant failed to meet protocol entry criteria or did not adhere to protocol requirements, excluding prolonged interruption of study drug.

c. Voluntary Withdrawal: The participant or legally authorized representative wishes to withdraw from the study. The reason for withdrawal, if provided, will also be documented in the CRF.

d. Other: This category includes participants withdrawn for occlusion of an IV or loss ofIV access.

6.8 Procedures for Discontinuation or Withdrawal of a Participant

If a participant is withdrawn from treatment and/or the study because of a clinically significant serious adverse event (SAE), the investigator will evaluate the urgency of the event. For urgent situations, the investigator will proceed with immediate treatment of the SAE and the participant's condition according

to standard of care. The IRB will be notified of any clinically significant, unexpected, related SAE leading to the participant's withdrawal from the study and/or required treatment.

The investigator may terminate participation of a subject for safety if he or she believes that continued participation in the study would put the participant's health at unacceptable risk. In addition, a participant or legally authorized representative may discontinue his or her participation without giving a reason at any time during the study.

7.0 STUDY PLAN

7.1 Definition of Study Procedures (see Appendix A: Schedule of Events)

7.1.1 Demographics, Medical History, and Medication History

Demographics will include the age, gender, ethnicity, and race as described by the participant or participant's legally authorized representative. A complete medical history will include a review of all major body systems. All concurrent medical conditions will be recorded. Medication history will include all medications taken or administered within 72 hours of study drug administration.

7.1.2 Vital Signs Excluding Hemodynamics

Vital signs will be collected and recorded on the CRFs. For this study, vital signs will include temperature, heart rate (pulse), and respiratory rate. Blood pressure will be considered a hemodynamic assessment in this study.

7.1.3 Hemodynamic Assessments

Blood pressure measurements will represent a major component of the hemodynamic assessments. Vasopressor dosages in critically ill patients will also form a significant component of these assessments. Vasopressor Dependency Index (VDI) will be used as the measure of hemodynamic assessment. VDI is a continuous variable calculated by dividing vasopressor index (summation of all pressors) by MAP. VDI will be calculated at baseline (immediately before start of the infusion), again 2 hours after completion of the bolus, and every 4 hours during the infusion. Any cases where the VDI increases by more than 20% from baseline will result in interruption of the study infusion and review by the safety officer in an expedited fashion.

7.1.3.1 Blood Pressure Measurements

Blood pressure measurements will be taken from an arterial catheter if available and sphygmomanometer if an arterial catheter is not available. If a participant has an arterial catheter in place, the MAP measured by both the arterial catheter and the sphygmomanometer will be documented in case the arterial line is discontinued and not replaced during the study.

7.1.4 Mortality, Hospital, and ICU Length of Stay Assessment

Participant survival will be assessed at hospital discharge. ICU length of stay will be the total number of days the participant spends in the ICU from time of enrollment to hospital discharge. Re-admissions to the ICU during the initial hospital stay will be added to the initial ICU length of stay to determine a total number of ICU days. Hospital length of stay will be calculated as the number of days from enrollment to

hospital discharge. Once the patient is discharged from the hospital (to any disposition including home, home with help, rehab, or another hospital) or dies, hospital days will cease. Subsequent re-admissions to the hospital or ICU after hospital discharge will not be added to the hospital or ICU lengths of stay, respectively.

7.1.5 Clinical Laboratory Tests

Hematology and serum chemistries, including basic metabolic panels, liver function testing, complete blood counts, and platelet levels will be performed as part of the participant's standard medical care at clinical care laboratories at the treating site. Five milliliters of blood for special laboratory measurements, including serum citrulline and arginine levels will be collected at each of the scheduled time points (see Appendix A) separated into plasma and cellular components and frozen at –70ºC. These samples will be thawed, and the variables measured, all at the same time, after all enrolled participants have completed the study. Citrulline and arginine will be measured using a Beckman 7300 Amino Acid Analyzer or equivalent. Safety labs, including complete blood count, complete platelet count, basic metabolic panel, and liver function tests, will be performed as part of the participant's standard medical care at clinical care laboratories at the treating site.

Five milliliters of blood will be collected in an EDTA anti-coagulated tube at hours 0, 2, 12, and every 2 days until extubation for a maximum of 10 days- i.e. days 1 (hours 0, 2, 12) then on days 2, 4, 6, 8, and 10. Plasma will be centrifuged and the supernatant and cellular portions will be frozen at –70ºC.

7.1.6 Pregnancy

Female participants of childbearing potential must have a negative urine or serum HCG pregnancy test to be eligible for the study.

7.2 Schedule of Observations and Procedures

The schedule for all study related procedures and evaluations is summarized in Appendix A.

7.2.1 Baseline Assessments

Eligibility of participants will be assessed based on the inclusion and exclusion criteria. Prior to initiation of study drug infusion (defined as study time=0 hours), baseline safety assessments, including complete blood and platelet counts, measures of renal and liver function, and metabolic panel, will be collected from the medical record. Measurements within 36 hours prior to starting study infusion will be considered appropriate for baseline measurements. In cases where more than one measurement is available in that 36 hours, the measurements closest to the start of study infusion will be used. Blood for baseline plasma citrulline and arginine will be drawn as close to time=0 as possible. Baseline hemodynamic measurements (i.e. MAP and vasopressor dosage) will be taken within 15 minutes prior to initiation of study material.

7.2.2 On Study (days 0-DC) Assessments

Study drug administration should begin as soon as possible after confirming the participant meets the eligibility criteria. Time=0 hours is defined as the time when study infusion is initiated. All times are calculated in relation to the time study drug was initiated at Hour 0. The study infusion will last for a maximum of 10 days.

MAP will be measured per hospital unit or ICU protocol during the entirety of the infusion. MAP and if required vasopressor dosages will be recorded in the CRF 2 and 12 hours after start of study infusion and as close as possible to 8AM on study days one through 10. VDI will also be calculated for these timepoints.

Blood samples for plasma citrulline, arginine measurements will be collected as close to 8AM as possible on study days 2, 4, 6, 8, and 10, after Hours 0, 2, 12 are completed. All of these specimens will be centrifuged, and the supernatant and cellular portions frozen at –70ºC for subsequent batch analysis of all specimens once all participants have completed the study.

Patients will discontinue study drug on Day 10. Patients will continue to have all procedures in the schedule of procedures except for screening procedures, study drug, and blood draw for plasma citrulline.

Patients who complete study visit day 11 will continue to be followed from day 12 to discharge. From day 12 to discharge the following values will continue to be collected during this time are vital signs, hospital status, ICU status, and non-invasive and invasive ventilator settings. A further follow up will occur on D28 and D60 as a telephone visit, if discharged earlier.

Concomitant medications and adverse event monitoring will be performed and reported in CRFs.

See the Schedule of Procedures in Appendix A for further details.

7.2.3 Clinical Outcome Assessments

ICU length of stay will be calculated in days from time = 0 to the earlier of ICU discharge or death. Any re-admissions to the ICU during the hospital stay will add days to ICU length of stay. Hospital length of stay will be calculated in days from time = 0 until the earlier of hospital discharge or death. A follow up at D28 and D60 will further evaluate the stated clinical outcomes.

Concomitant medications and adverse events will continue to be monitored and recorded in the CRF for the duration of the participant's hospital stay.

See the Schedule of Procedures in Appendix A for further details.

8.0 PROTOCOL DEVIATIONS

Protocol deviations will be recorded and reported to the local IRB. If the protocol deviation has placed the participant at increased risk, it will be reported to the safety officer and the IRB within 24 hours. If the protocol deviation has resulted in no increased risk, the event will be reported to the IRB at continuing review.

9.0 ADVERSE EVENTS

Investigators will determine daily if any clinical adverse experiences occur during the period from enrollment to 48 hours after study drug termination. Most data normally collected as part of a clinical study to guarantee patient safety and determine efficacy is obtained as part of normal clinical care of patients in the hospital or ICU. Examples include arterial blood gases, electrolytes, liver function tests, hemograms, and chest radiographs. All available clinical data, including radiographs, laboratory values, vital signs, exam findings, and clinical impressions from the primary team, will be monitored for adverse events. The investigator will evaluate any changes in laboratory values and physical signs and will

determine if the change is clinically important and different from what is expected during treatment of patients with acute hypoxemic respiratory failure. If clinically important and unexpected adverse experiences occur, they will be recorded on the adverse event case report form. Investigators will report all serious and unexpected adverse events to the safety officer and the local IRB in a timely manner. All adverse events that are deemed non-serious and or not unexpected will be reported to the IRB annually at continuing review.

Patients will be closely monitored in a hospital or ICU setting. This will allow for prompt treatment of any adverse event that is suspected to be related to study drug, in which case the safety monitor will be notified and will evaluate the situation. In such an instance study drug infusion will be withheld until the event is evaluated and a decision has been made whether the infusion will be restarted or permanently discontinued.

9.1 Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product that does not necessarily have to have a causal relationship with this treatment. An adverse event therefore can be any unfavorable and unintended sign (e.g., abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not the incident is considered to be related to the investigational product. Incidences of hypotension, hepatitis (i.e. elevation of liver enzymes), and injection site reactions will be systematically collected and will be considered adverse events.

9.2 Clinically Significant Laboratory Values

Any change in laboratory values will be evaluated. If a laboratory abnormality is new from baseline and thought to be clinically significant, it is considered a laboratory adverse event. However, if the laboratory value abnormality is consistent with a current diagnosis, it will not necessarily be considered an adverse event. Changes in laboratory values will only be considered adverse events if they are judged to be clinically significant (if some action or intervention is required or if the investigator judges the change to be beyond the range of normal fluctuation). Due to most of the urea cycle enzymes being hepatically located, elevation of liver enzymes will be systematically collected.

9.3 Pre-Existing Conditions

Pre-existing conditions that are present before the start of the study infusion will be considered concurrent medical conditions and should NOT be recorded as adverse events. However, if the subject experiences a worsening or complication of such a concurrent condition, the worsening or complication may be recorded as an adverse event at the discretion of the investigator.

The following are clinical outcomes of an acute hypoxemic respiratory illness progressing to critical illness due to COVID-19 infection (SARS-CoV2), and as such, will not be considered as adverse events:

1. Respiratory: decreased SaO2/FiO2 or PaO2/FiO2, mechanical ventilation, acute lung injury or acute respiratory distress syndrome (ARDS), or respiratory failure
2. Hepatic: hepatic injury or liver dysfunction; increased bilirubin
3. Renal: renal failure, need for dialysis, increased creatinine
4. Coagulation: disseminated intravascular coagulation (DIC), thrombocytopenia
5. Central Nervous System (CNS): acute mental status changes (not associated with localizing neurological signs)

6. Vital Signs: tachypnea, hypothermia, hyperthermia, tachycardia

Cardiovascular function will be systematically collected as both MAP and vasopressor dosages. VDI will be calculated at baseline (immediately before start of the infusion), again 60 minutes after completion of the bolus, and every 4 hours during the infusion. Any cases where the VDI increases by more than 33% from baseline will be considered an adverse event and submitted for review by the safety officer in an expedited fashion.

9.4 Serious Adverse Events

A serious adverse event (SAE) is defined as any untoward medical occurrence that meets any of the following criteria:

a. Results in death
b. Is life-threatening (defined as an event in which the participant was at risk of death at the time of the event and NOT an event that hypothetically might have caused death if it had been more severe)
c. Requires inpatient hospitalization
d. Prolongs an existing hospitalization
e. Results in persistent or significant disability or incapacity
f. Results in a congenital anomaly or birth defect
g. Important medical event that requires an intervention to prevent any of a-f above.

h. SAE qualifying as a SUSAR (serious unexpected suspect adverse reaction) willbe subject to expedited reporting as per safety plan.

9.5 Adverse Event Collection Period

Collection of adverse events will commence from the time that the participant is first administered the study infusion (study hour =0). Routine collection of adverse events will continue until day 10. Any adverse events spontaneously reported to the investigator or observed by the investigator after day 10 will also be collected and recorded on the CRF.

10.0 STATISTICAL METHODS

10.1 Analysis Populations

The intent-to-treat (ITT) population will be used for all efficacy and safety analyses. This will consist of all randomized patients irrespective of whether the patient received study drug or the patient's compliance with the study protocol, in the treatment group assigned by the randomization system.

10.2 General Statistical Approach

Before database lock, a statistical analysis plan (SAP) will be issued as a separate document, providing full detailed methods for the analyses outlined below. Multiplicity adjustment approach will be described as per industry guidance for multiple comparisons across all the endpoints to eliminate Type 1 errors. Sensitivity analysis will be applied to handle missing data and sources of uncertainty. The protocol study team may revise the plan during the study to accommodate clinical trial protocol amendments and to make changes to adapt to unexpected issues in study execution and data that affect planned analyses. The protocol team will conduct all statistical analyses following the statistical principles for clinical trials as specified in International Council on Harmonization Topic E9. Any deviations from the planned analyses

will be described and justified in the final integrated clinical study report. The protocol study team will present overall and treatment specific data and summary tables.

All statistical tests will be two-sided. All summary tables for quantitative measures will display means, standard deviations, median and range, as well as number of missing data (if relevant). All summary tables for categorical variables will display counts, percentages, and number of missing data (if relevant).

10.3 Demographics and Baseline Characteristics

Demographics and baseline characteristics will include age, gender, race, comorbidities, baseline vital signs and laboratories. Descriptive statistics, including mean and standard deviation, median, intra-quartile ranges, minimum and maximum, and the number and percent of subjects in specified categories will be used to summarize the demographic and baseline variables for the two study arms. These will be compared between groups using independent two-sample test or Wilcoxon rank-sum test, as appropriate, for continuous variables and Fisher's Exact Test for categorical variables. All statistical analyses will be done using SAS statistical software.

10.4 Sample Size

This study will accrue approximately 66 patients in a 1:1 fashion to account for 10% attrition. This sample size was determined on the basis of an expected time to intubation event in the placebo group derived from historical data on critically ill patients with COVID-19 infection (SARS-CoV-2) of 3 days (72 hours) +/- 3 days (72 hours). An acceptable effect size in the intravenous citrulline group would be delay in time to an intubation event of at least 6.5 days (156 hours). Thus, a two-sided log-rank test with an overall sample size of 60 patients achieves 81% power at a 0.05 significance level to detect a hazard ratio of 0.46 when the placebo group time to intubation is 3 days. The study is expected to last 12 months, of which patient accrual will occur in the first 6-9 months. The accrual pattern across time is uniform.

In terms of mortality, in patients who progress to acute hypoxemic respiratory failure requiring ICU care, the mortality is 50%. With a sample size of 60 patients, the study will have 80% power to detect a difference between the group proportions of -33%. The mortality in the intravenous citrulline group is assumed to be 50% under the null hypothesis and 17% under the alternative hypothesis. Again, the mortality rate for the placebo group is assumed to be 50%. The test statistic used is the two-sided z-test with pooled variance with a 0.05 significance level.

10.5 Primary Endpoints

Time to an intubation event in hours from the start of the study drug infusion or overall duration of mechanical ventilation will represent the primary clinical endpoint. The number of patients starting intubation over time will be displayed in the form of survival curves using the Kaplan-Meier method and analyzed using a two-sided log-rank test. Statistical significance will be claimed if the computed p-value is equal to or less than 0.05.

Citrulline and arginine plasma levels will represent the primary biochemical endpoint of this study. A 5 mL sample of blood will be obtained for amino acid analysis at hour 0 before study medication is administered, 2 and 12 hours after the bolus and as close as possible to 8 AM on days 2, 4, 6, 8, and 10. Samples will be collected in citrated tubes and placed on ice until they can be centrifuged. Plasma and cellular components will be frozen at –70 ºC until batch analysis. Concentrations of citrulline, arginine, and ornithine will be measured in the plasma samples using cation-exchange chromatography with a Beckman 7300 amino acid analyzer or equivalent. The analysis team will provide the means and standard

deviations for citrulline, arginine, and ornithine plasma levels separately for each treatment group. A generalized linear mixed model (GLMM) with appropriate link function (i.e., identity link for continuous outcome) will be used to compare these plasma amino levels across the two intervention groups. The model will examine how the treatment means differ (i.e., main treatment effect), how treatment means change over time (i.e., main time effect), and how differences between treatment means change over time (i.e., treatment-by-time effect).

The primary safety outcome will be worst VDI any time after start of infusion. VDI is a continuous variable calculated by dividing vasopressor index (summation of all pressors) by MAP. VDI will be calculated at baseline (immediately before start of the infusion), 2 hours after completion of the bolus, and every 4 hours during the infusion. Worst VDI will be compared between groups using ANCOVA analysis with the baseline VDI as the covariate.

10.6 Secondary Safety Endpoints

Although VDI will represent the primary outcome for determining safety, other measures will also be employed.

10.6.1 Secondary Clinical Endpoints

All-cause, hospital mortality, defined by whether the patient was discharged from the hospital alive or deceased, will be analyzed via two statistical methods. The proportion of patients who have died by hospital discharge will be analyzed using a Chi-square analysis or Fisher's Exact Test, if needed. In addition, time to death will also be analyzed using a log-rank method of a Kaplan-Meier analysis. ICU length of stay (LOS) will be defined in days as the time from study enrollment to the day ICU discharge orders are written, regardless of when the patient physically leaves the ICU. Comparison will be made between the two groups for total ICU length of stay which will include any days of ICU re-admission prior to hospital discharge. Hospital length of stay will be defined as time from study enrollment to first hospital discharge. We will consider both ICU LOS and hospital LOS as count measure and has the potential to follow a skewed distribution. Initially, we will assess the distributional assumption. We will use a generalized linear model (GLM) to compare the expected ICU LOS and hospital LOS between the two treatment groups (placebo and intravenous citrulline). Specifically, we will use a negative binomial distribution and log-link to account for potential over-dispersion. We will report point estimates for the group mean difference along with a 95% confidence interval. Overall duration of mechanical ventilation will be modeled with zero-inflated Poisson or negative binomial method using consent and post-infusion start time for two separate analysis.

10.7 Interim Analyses

No formal interim analyses for efficacy will be done in this phase 2 study. A safety analysis looking at VDI and hypotension will be undertaken after the first 20 patients have completed enrollment to ensure that citrulline administration is not causing hypotension.

11.0 ETHICS

11.1 Ethical Conduct of the Trial

This study will be conducted with the highest respect for individual participants according to the protocol, the World Medical Association Declaration of Helsinki, the Guideline for Good Clinical Practice, and the

Belmont Report. Furthermore, the study will be conducted in accordance with the Common Rule (45 CFR 46), as guided by the Department of Health and Human Services via the Office of Human Research Protections.

11.2 Institutional Review Board

The investigator and sponsor will submit all relevant documents to the IRB for the protocol's review and approval. The study, protocol, informed consent form, investigator's brochure, and any proposed advertising or recruitment materials must be approved by the local IRB prior to commencing the study. Written approval by the IRB of the protocol and participant informed consent document must be obtained prior to enrollment of any participants. If any member of the IRB has direct participation in this trial, he or she must refrain from any IRB discussions or votes pertaining to this study.

Sites must adhere to all requirements stipulated by their respective IRBs. This may include notification to the IRB regarding: protocol amendments, updates to the informed consent document, recruitment materials, local safety reporting requirements, and closure of the study.

11.3 Participant Information, Informed Consent, and Participant Authorization

All consent for participation in this study must occur via written consent documents, which should contain the elements of informed consent as required by 45 CFR 46 and the Declaration of Helsinki. The informed consent document will describe the planned and permitted uses, transfers, and disclosures of the participant's personal and personal health information for purposes of conducting the study.

The investigator is responsible for the preparation, content, and submission to the IRB approval of the informed consent form. The informed consent form and any participant information sheet must be approved by the IRB prior to use.

The participant, or his or her legally authorized representative, must be presented with an informed consent document written in their native language and given ample opportunity to: 1) inquire about details of the study, and 2) decide whether or not to participate in the study. If the participant or legally authorized representative agrees to participate in the study, the informed consent form must be signed and dated by the participant or legally authorized representative prior to the participant being enrolled in the study. The investigator must document the date the ICF is signed in the participant's medical record. The investigator must also sign and date the informed consent form at the time of consent and prior to the participant being entered into the study.

Once signed by both the participant and investigator, a copy of the informed consent document will be given to the participant (or legally authorized representative). The original informed consent document will be stored in the investigator's site file. All revised informed consent forms must be reviewed and signed in the same manner as the original informed consent. The date the revised consent was obtained should be recorded in the participant's medical record and the participant should receive a copy of the revised ICF. If surrogate consent was utilized for initial consent, the participant should be "re-consented" by signing the original ICF when he or she regains the capacity to consent. The participant should date this "reconsent" with the actual date of reconsenting and not with the date of the original consent signing by the legally authorized representative.

11.4 Data and Safety Monitoring Plan

The Principal Investigator, along with the Chief Medical Officer at Asklepion (safety officer) will be responsible for overseeing the safety of this trial. There is no formal data safety monitoring board planned for this trial. Laboratory and clinical data will be reviewed daily for adverse events by the safety officer. In addition, the safety officer will be available at any time for questions from the co-investigators at UAMS, who will be monitoring the patients continuously for potential adverse events. There will be no interim analyses of the data for efficacy with the exception of an initial independent PK evaluation (separate from study team) of first 2 subjects for all the time points to ensure steady state concentration is being attained The safety officer will be available to review serious adverse events in a timely manner. Furthermore, in cases of serious adverse events or significant hypotension, the safety officer can request unblinding of the treatment arm and/or an urgent amino acid analysis to better guide decision-making. The safety officer will have the ability to pause the trial in agreement with the co-investigators to enable investigation of possible safety issues and/or suggest changes to the design of the study to abrogate any safety issues, including discontinuation of the initial bolus should it result in unacceptable hypotension.

11.5 Potential Risks

All patients will reside in a hospital unit or intensive care unit environment, where close monitoring can be achieved and rapid response to any potential adverse event, including hypotension, will occur. Should a patient improve and be weaned off all oxygen support prior to completion of the study drug infusion, the study drug will be stopped but data will continue to be collected. Patients will be monitored by the bedside or ICU nurse for the duration of the infusion per standard unit protocol. The investigator and/or coordinator will be available by pager 24 hours a day 7 days a week to answer any questions that the bedside nurse has.

All patients will have blood drawn for research purposes. The risks of drawing blood are uncommon and may include bleeding and bruising. Commonly, having blood drawn is painful, and rarely can lead to infection at the site of the blood draw. It is anticipated that most of the blood obtained for research purposes will be obtained through existing intravenous peripheral, central, or arterial catheters since these patients routinely have such catheters while in the intensive care units. The amount of blood drawn is minimal and represents a small percentage of the amount of blood taken during a standard hospital or ICU stay and will not represent a significant risk to the patient.

The risks of citrulline are theoretical. In previous studies enrolling hundreds of humans, no serious adverse events have been attributed to either oral or intravenous citrulline. Citrulline may increase NO production and exacerbate hypotension. Arginine, a product of citrulline via the urea cycle, has been reported to exacerbate hypotension. The blood pressure of every participant in this study will be monitored closely throughout the study and especially during the infusion. As these patients will be cared for in a hospital unit or ICU, blood pressure measurements will be taken per hospital unit or ICU protocol. Hypotension, as measured by vasopressor dependency index (VDI) represents an *a priori* endpoint of the study. VDI will be calculated 2 hours after the bolus and daily. Any worsening of VDI by 33% or more will prompt review by the safety monitor. Although it is likely to reduce organ dysfunction by attenuating inflammation and oxidative stress, daily labs will be monitored for adverse events on organ functioning. The citrulline will be administered in a small volume of 5% dextrose water (30 mL bolus followed by ~15 mL/hr). This small amount of dextrose should not dramatically increase blood sugar levels in these patients and as such, should pose minimal risk. Furthermore, any event which the investigator or primary team is concerned may be related to study drug administration will be reported as an adverse event. Participants will be monitored for any signs of allergic reaction, including new wheezing, itching and/or rash. Blood pressure, which might fall during severe allergic reactions such as anaphylaxis, will be closely monitored in the hospital or ICU setting with treatment available as needed.

The placebo will be a small volume of 5% dextrose water (30 mL bolus followed by ~15 mL/hr). Although administration of dextrose in theory could raise blood sugar levels, the small volume will result in little dextrose being administered which should not significantly increase blood sugar levels in these patients and as such, should pose minimal risk. In addition, blood sugar levels are routinely measured and monitored closely as part of routine care in intensive care unit patients, especially those with severe sepsis.

Another potential risk to participants is the breach of confidentiality including information from their medical record. There are many safeguards in place to prevent the release of information from this study. All data for this study will be stored in a password-protected, HIPAA security compliant computer database. Initially it will be recorded on case report forms which will be stored in a locked office that only the study personnel have access to. This is especially true since some genetic tests will be run utilizing these samples. All samples obtained for this study will be assigned a code which will not identify the subject. The only key to the code will be in the password-protected computer database. Dr. Meena, and the study co-investigator will have access to the code and information that identifies a participant as a being in this study. The results of analyses run on those samples will not be reported to the participant or primary study team. No one else, including relatives, doctors, employers, or insurance companies will be allowed to view the test results. The codes linking the patients with their data will be destroyed after all participants have completed the study and the data has been through quality assurance processes. Informed consent documents will not bear the study identification number so that once the code is destroyed; there will be no possible way the results could be traced back to the individual.

11.6 Alternative Treatments

There are no alternative treatments. Patients who are participating in another interventional study will be ineligible for this study. Participants will be told they have the option to choose not to participate in this study. The primary team will manage the participants' clinical condition according to their best clinical judgment. Any potential treatments for acute hypoxemic respiratory illness, including low tidal volume ventilation in patients requiring mechanical ventilation will be allowable, determined by the primary medical team, and not restricted by participating in this study. The blinded study drug material will be added to the treatment course that the primary team considers appropriate.

11.7 Potential Benefits

The potential benefits to science and mankind that may result from this study include the knowledge that we will obtain regarding the effects of citrulline and the urea/NO cycle on inflammation and oxidative stress in an acute hypoxemic respiratory illness. In addition, we will also gain valuable information regarding the safety of IV citrulline in COVID-19 (SARS-CoV2) infected patients. Since the urea/NO cycle and NOS play an integral role in redox homeostasis, administration of citrulline may decrease oxidative stress and improve organ function, especially pulmonary function, even potentially mitigating acute lung injury in these patients. The patients randomized to the placebo arm will not directly benefit from participating in this study but will provide vital information for the interpretation of the study results and a valuable contribution to medical science.

These benefits are reasonable in relation to the relatively few known risks of L-citrulline. L-citrulline, by re-establishing urea/NO cycle homeostasis and reducing oxidant stress, may improve clinical outcomes, including mitigation of organ dysfunction, particularly lung injury. These improved outcomes are desirable by all patients seeking medical attention. L-citrulline has been used in humans previously and has been shown to be safe.

12.0 DATA HANDLING AND RECORDKEEPING

12.1 Sources of Research Material

All materials will be collected and recorded for research purposes. The data collected during this investigation will be obtained from multiple sources, including medical records, observational data, and serum samples. One of the main sources of data will be chart abstraction utilizing the patient's medical record. The progress notes and bedside nursing notes and daily charting will also be used to collect data. Interviews with patients and/or patients' family will be conducted both in person and over the phone on occasion. The patients will have serum samples collected at the specified timepoints for specialized laboratory analyses. All the remaining data will be derived from the interactions with the patient in the form of the nurses' and physicians' examinations and interviews.

12.2 Participant Confidentiality

The investigators affirm and uphold the principle of the participant's right to protection against invasion of privacy. Throughout this study, a participant's source data will only be linked to the clinical study database or documentation via a unique study identification number. As permitted per HIPAA regulations, limited participant attributes such as sex, age, or gender may be used to verify the accuracy of the participant's unique identification number. Copies of any participant source documents provided to entities outside of the University of Arkansas for Medical Sciences (including the FDA, IRB, or NIH) will have all personally identifiable information removed (e.g., participant name, address, date of birth)

The research coordinator, principal investigator, or co-investigators will collect data and record it on case report forms designed specifically for the study. The data will then be entered in a password protected, privacy-secured, computer database. The data collection forms will be kept in a locked cabinet in a locked office at each site. Only the study investigators and study coordinators will have access to the locked cabinet. All samples obtained for this study will be assigned a code which will not identify the subject. The only key to the code will be in the password-protected computer database. The Principal Investigator will be responsible for maintaining confidentiality of data and codes. Only study investigators and the study coordinator will have access to the code and information that identifies a volunteer as a being in this study. The results of analyses run on those samples will not be reported to the participant or primary team. The codes linking the patients with their data will be destroyed after all participants have completed the study and the data has been through quality assurance processes.

12.3 Case Report Forms

CRFs will be completed for each participant randomly assigned to study medication or placebo. CRFs will be completed in legible black ink and will be written in English. Corrections will be made via a single line strikeout of the incorrect information and writing in the revisions. All corrections must be initialed and dated. The completed original CRFs are the sole property of site where enrolled.

12.4 Medrio Computerized Database

Medrio EDC will be used as the data capture tool. It is a secure, cloud-based application that provides 1) intuitive navigation for validated data entry; 2) detailed audit trails for tracking data manipulation (e.g. old/new/deletion, approvals, locking); 3) reporting modules and on-demand data extraction capabilities; and 4) tools for importing data from external sources.

Data within Medrio is siloed and independently structured to ensure that a study and its users can access only their own study. Thus, only study personnel for this project with appropriate permissions will be allowed to access the database. Authorized users can input data into Medrio from anywhere in the world with secure web authentication and data logging.

12.5 Record Retention

The investigator will keep all study-specific documents, an identification log of all participating patients, source worksheets, original signed and dated informed consent documents in a locked and secure office or storage space until at least three years after the completion of the study. In addition, the secure Medrio database will be maintained for at least three years after completion of the study. The data will be housed with the sponsor from the Medrio EDC.

13.0 REFERENCES

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14. Investigator Signature Page

Protocol Title: Prospective, Randomized, Double-Blind, Placebo-Controlled Phase II Trial of Intravenous L-Citrulline to Delay and Potentially Prevent the Need for Invasive Mechanical Ventilation for Acute Hypoxemic Respiratory Failure in Patients with COVID-19 (SARS-CoV-2) Illness

Protocol Number: CIT-COVID19-002-01 Version 3.0 Dated 11 June 2021

Confidentiality and cGCP Compliance Statement

I, the undersigned, have reviewed this protocol, including appendices and I will conduct the study as described in compliance with this protocol, GCP, and relevant ICH guidelines.

Once the protocol has been approved by the IRB/IEC, I will not modify this protocol without obtaining prior approval of the Asklepion Pharmaceuticals, LLC and of the IRB/IEC. I will submit the protocol modifications and/or any ICF modifications to Asklepion Pharmaceuticals, LLC and IRB/IEC, and approval will be obtained before any modifications are implemented.

I understand that all information obtained during the conduct of the study regarding the subjects' state of health will be regarded as confidential. No subjects' names will be disclosed. All subjects will be identified by assigned numbers on all Case Report Forms, laboratory samples or source documents forwarded to the sponsor. Clinical information may be reviewed by the sponsor or its agents or regulatory agencies. Agreement must be obtained from the subject before disclosure of subject information to a third party.

Information developed in this clinical study may be disclosed by Asklepion, to other clinical investigators, regulatory agencies, or other health authority or government agencies as required.

DocuSigned by:

Signer Name: Nikhill K. Meena, MD
Signing Reason: I approve this document
Signing Time: 15-Jun-2021 | 5:03:59 AM PDT
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15-Jun-2021

Investigator Signature — Date

Nikhill K. Meena, MD

Printed Name

UAMS

Institution

APPENDIX A: Schedule of Events

Measurement	Day 0 (baseline)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12-DC	Day 28 [e] Follow-up	Day 60 [e] Follow-up
Informed Consent	X														
Demographics- Hx & Px	X														
Eligibility Assessment	X														
Pregnancy Test	Xc														
Study Drug Administration		X	X	X	X	X	X	X	X	X	X				
Vital Signs		X	X	X	X	X	X	X	X	X	X	X	X		
Mean Arterial Pressure		Xa,b,d	X	X	X	X	X	X	X	X	X	X			
Vasopressor Dosages		Xa,b,d	X	X	X	X	X	X	X	X	X	X			
Basic Metabolic Panel		A	A	A	A	A	A	A	A	A	A	A			
CBC		A	A	A	A	A	A	A	A	A	A	A			
LFTs		Ab	A	A	A	A	A	A	A	A	A	A			
D-dimer blood draw		Xa,b	X		X		X		X		X		X		
CRP		Xa,b	X		X		X		X		X		X		
5 ml blood for citrulline etc.		Xa,b	X		X		X		X		X				
SaO2/ FiO2 ratio		X	X	X	X	X	X	X	X	X	X	X			
Pa02		X	X	X	X	X	X	X	X	X	X	X			
ABG		A	A	A	A	A	A	A	A	A	A	A			
Hospital status		X	X	X	X	X	X	X	X	X	X	X	X	X	X
ICU status		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Non-invasive ventilation status		X	X	X	X	X	X	X	X	X	X	X	X		
Invasive ventilation status		X	X	X	X	X	X	X	X	X	X	X	X		
ConMed Review		X	X	X	X	X	X	X	X	X	X	X	X	X	X

Adverse Event Review		X	X	X	X	X	X	X	X	X	X	X	X	X	X

a= measurement should be recorded or performed at hours 0, 2 and 12
b= Day 1 hour 0 samples/procedure must be collected/recorded before study drug is administered.
c= only perform if patient is of childbearing potential
d= must be collected 15 minutes prior to administration of study medication
e= Day 28 and Day 60 are telephone follow ups
X= Study Procedure A= Standard of Care

APPENDIX B- Protocol Clarification Memo

MEMO TO FILE

DATE: 20 January 2021

STUDY NUMBER: CIT-COVID19-002-01

STUDY TITLE: Prospective, Randomized, Double-Blind, Placebo-Controlled Phase II Trial of Intravenous L-Citrulline to Delay and Potentially Prevent the Need for Invasive Mechanical Ventilation for Acute Hypoxemic Respiratory Failure in Patients with COVID-19 (SARS-CoV-2) Illness

Protocol Version/Date: Version 2.0, 08 September 2020

RE: Protocol Clarification Memo: Requirements of Artiral Blood Gas (ABG) Timepoint

The diagnostic criteria for acute lung injury (ALI) and ARDS utilize the Pao(2)/fraction of inspired oxygen (Fio(2)) [P/F] ratio measured by arterial blood gas analysis to assess the degree of hypoxemia. We hypothesized that the pulse oximetric saturation (Spo(2))/Fio(2) (S/F) ratio can be substituted for the P/F ratio in assessing the oxygenation criterion of ALI/ARDS associated with SARS Cov2.

S/F ratios correlate with P/F ratios. S/F ratios of 235 and 315 correlate with P/F ratios of 200 and 300, respectively, for diagnosing and following up patients with ALI and ARDS.

UAMS Standard Of Care (SOC) protocol restricts arterial sample collection for ABGs and hence the need to issue the memo.

The ABG requirement in the protocol is not required but if the data is collected it will be entered into the Clinical Data Base.

DocuSigned by:
Signer Name: Gurdyal Kalsi
Signing Reason: I approve this document
Signing Time: 20-Jan-2021 | 2:29:19 PM PST
87C0D7E826E7451AAFB13CB3307C2ED1

Gurdyal Kalsi, MD MFPM (Hon)
Chief Medical Officer
Asklepion Pharmaceuticals, LLC

20-Jan-2021
Date

Protocol Clarification Memo Version 1.0 dated 20Jan2021 Final

APPENDIX C: Protocol Clarification Memo- Procedure Window Allowance

MEMO TO FILE

DATE: 03 March 2021

Protocol Number: CIT-COVID19-002-01

Protocol Title: Prospective, Randomized, Double-Blind, Placebo-Controlled Phase II Trial of Intravenous L-Citrulline to Delay and Potentially Prevent the Need for Invasive Mechanical Ventilation for Acute Hypoxemic Respiratory Failure in Patients with COVID-19 (SARS-CoV-2) Illness

Protocol Version/Date: Version 2.0, 08 September 2020

RE: Protocol Clarification Memo- Procedure Window Allowance

During the trial's conduct, the procedures outlined below warrant an allowable window around the scheduled timepoint. These windows also allow for real world experience in the clinical trial.

1.0 PK/PD Blood Sample Collection:

Timepoint:	Procedure Window:
Hour 0	No Window
Hour 2	± 30 Minutes
Hour 12	± 30 Minutes
Day 2	± 30 Minutes within 24 hours from Hour 0 timepoint
Day 4	± 2 Hours
Day 6	± 2 Hours
Day 8	± 2 Hours
Day 10	± 2 Hours

2.0 PK/PD Blood Volume Clarification:

The minimum blood volume needed for the PK/PD analysis requires a minimum of 5 mL of whole blood. Given the tube sizes available, it is not possible to draw 5 mL of blood given that the required tube comes in 3 mL or 7 mL volume. If a sample should have more than 5 mL of blood drawn, it will not be considered a deviation. However, any blood volume under 5 mL will be recorded as a deviation as it may not provide enough plasma to run the assay. All samples will be processed even if the volume is not 1 mL plasma.

3.0 CRP and D-dimer Blood collection:

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CRP and D-dimer blood collections will have an allowable procedure window of ± 30-minutes from the scheduled blood collection time. Please see the table below. There will not be a procedure window for hour 0 blood collection.

Timepoint:	Procedure Window:
Hour 0	No window
Hour 2	± 30 Minutes
Hour 12	± 30 Minutes
Day 2	± 30 Minutes within 24 hours from Hour 0 timepoint
Day 4	± 30 Minutes
Day 6	± 30 Minutes
Day 8	± 30 Minutes
Day 10	± 30 Minutes

4.0 Follow-up Visit Day 28 and Day 60:

Timepoint:	Procedure Window:
Day 28 Follow-up	± 2 Days
Day 60 Follow-up	± 2 Days

The above procedure windows are allowed as this will allow for real-world evidence and not impact the analysis.

DocuSigned by:
Signer Name: Gurcyal Kalsi
Signing Reason: I approve this document
Signing Time: 03-Mar-2021 | 9:07:03 AM PST
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03-Mar-2021

Gurdyal Kalsi, MD, MFPM (Hon)
Chief Medical Officer
Asklepion Pharmaceuticals, LLC

Date

15. Summary of Changes:

SECTION	DESCRIPTION of CHANGES
1.0	Protocol Version Date
2.0 STUDY SUMMARY	Study Design-D28 and D60 telephone follow up
2.0 STUDY SUMMARY	Treatment Arms-added independent pharmacist
2.0 STUDY SUMMARY	Sample Size/Statistical Considerations-added PaO2
2.0 STUDY SUMMARY	Primary Biochemical Efficacy Variables-added surrogate marker info
2.0 STUDY SUMMARY	Secondary Biochemical Efficacy Variables-added dose proportionality info
4.1 Primary Endpoints (objectives)	Primary Endpoints Objectives-added association of plasma amino acids
5.1 Study Description	Study Description-added telephone follow up for D28 and D60
6.4 Informed Consent	Informed Consent-added PK Sample explanatory analysis
7.1.5 Clinical Laboratory Tests	Clinical Laboratory Tests-added timepoints
7.2.2 On Study (days 0-DC) Assessments	On Study Assessments-added timepoints
10.2 General Statistical Approach	Clinical Outcomes-added D28 and D60
9.3 Pre-Existing Conditions	Pre-Existing Conditions-added Sa02/Fi02
10.2 General Statistical Approach	General Statistical Approach-added multiplicity adjustment approach
10.4Sample Size	Data and Safety Monitoring Plan-added independent PK evaluation info
APPENDIX A: Schedule of Events	Schedule of Events-added footnotes to D28 and D60
APPENDIX A: Schedule of Events	Schedule of Events-added Pa02
APPENDIX A: Schedule of Events	Schedule of Events-added D-dimer test and CRP
APPENDIX A: Schedule of Events	Schedule of Events-added Conmed and AE review

Changes from Protocol Version 2.0 dated 08 September 2021 to Version 3.0 Dated 11 June 2021	
Section	**Description of Changes**
1.0	Protocol Version and date were updated to reflect Version 3.0 dated 11 June 2021 and added the IND number.
Table of Contents	The table of contents was updated to reflect the addition of Appendix B and C.
2.0 Study Summary	Updated Section "Sample Size/Statistical Considerations:" to reflect that up to 66 participants can be enrolled.
5.1 Study Description	The wording is updated to reflect. Up to 66 participants can be enrolled.
6.1 Participant Enrollment	The wording is updated to reflect up to 66 participants can be enrolled.
10.4 Sample Size	The wording is updated to reflect up to 66 participants can be enrolled. The wording was also updated to account for the additional patients in the analysis
14.0 Investigator Signature Page	Updated the protocol version and date.
APPENDIX	Protocol Clarification Memo: Requirement of Arterial Blood Gas (ABG) Timepoint was added (APPENDIX B), and the Protocol Clarification Memo-Procedure Window Allowance was added (APPENDIX C)