CIT-COVID19-002-01

Statistical Analysis Plan

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

SPONSOR:

Asklepion Pharmaceuticals, LLC

Baltimore, Maryland, USA

STATISTICAL ANALYSIS PLAN SIGNATURE PAGE

Reviewed and Approved by:

DocuSigned by:
Signer Name: Gurdyal Kalsi
Signing Reason: I approve this document
Signing Time: 16-Sep-2021 | 6:33:00 AM PDT
8750D76826FA481AAFB13CB0307C2ED1

16-Sep-2021

Gurdyal Kalsi, MD, MFPM
Medical Monitor
Chief Medical Officer
Asklepion

Date

Reviewed and Approved by:

DocuSigned by:
Signer Name: Vincent Elliott
Signing Reason: I approve this document
Signing Time: 16-Sep-2021 | 6:25:43 AM PDT
CE65BF711C094205B6DC50C2DE87A2B2

16-Sep-2021

Vincent Elliott
Associate Director of Clinical Operations
Asklepion

Date

Prepared, Reviewed and Approved by:

DocuSigned by:
Signer Name: Songthip T Ounpraseuth
Signing Reason: I approve this document
Signing Time: 16-Sep-2021 | 6:40:02 AM PDT
E8ADFBB5E19C49FAAA727803B181381F

16-Sep-2021

Songthip T. Ounpraseuth
Study Statistician
Independent Consultant

Date

Table of Contents

INTRODUCTION

This document outlines the planned statistical analyses for data collected within the scope of Asklepion L-Citrulline protocol CIT-COVID19-002-01, entitled "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". This statistical analysis plan (SAP) applies to the protocol v3.0, 11 June 2021. The purpose of this plan is to provide general, and in some instances, specific guidelines for which the various analyses will proceed. The primary analysis will include clinical efficacy, biochemical efficacy, and safety data up to the primary analysis data cut-off date.

OBJECTIVES

Primary Endpoints (objectives)

The primary objectives of the study are as follows:

- 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.

Secondary Endpoints (objectives)

The secondary objectives of this study are as follows:

- 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.
- To evaluate beneficial effect of intravenous L-Citrulline on hemodynamics.
- 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

STUDY DESIGN

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). Patients 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.

Approximately 60 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.

STUDY ASSESSMENTS

The assessment of efficacy will include collection of plasma for analysis of citrulline and arginine levels at 2 hours, 12 hours, 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, D-dimer tests, CRP 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.

TIMELINE/ASSESSMENT PERIOD

The schedule of events, appendix A, is described in the protocol v2.0, 08 September, 2020.

Data Base Lock is planned for September 2021

Analysis period will last September 2021 to October 2021.

CSR completion is planned for October to November period.

RANDOMIZATION

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.

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.

STUDY ENDPOINTS AND COVARIATES

Table 1: Primary and Secondary Efficacy Endpoints

Primary Clinical Efficacy Variables:	The primary clinical efficacy variable is time to intubation event in hours from the start of study infusion.
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
Secondary Biochemical Efficacy Variables:	1. Plasma levels of citrulline at 2 hours, 12 hours, and study days 2, 4, 8, 10. 2. Plasma levels of arginine at 2 hours, 12 hours, and study days 2, 4, 8, 10. 3. Plasma NOx levels at 2 hours, 12 hours, and study days 2, 4, 8, 10.

Table 2: Secondary Safety Endpoints

Safety Variables:	1. Primary safety variable is hemodynamic status, specifically worst vasopressor dependency index through day 10. 2. Incidence of reported adverse events.
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.

Table 3: Covariates

Independent Variables:	Age is likely to be an independent covariate and the intention is to assess its contribution in two age groups (younger and older) as per protocol.
Dependent Variables:	The outcome variable of interest being reduction in morbidity and mortality outcomes. A fuller list is as per the secondary endpoints above.
Continuous variable:	The study involves markers of respiratory function such as P/F ratios and hemodynamic markers such as VDI that are considered continuous variables and are expected to change with study treatment.

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.

SAFETY POPULATIONS

The Safety population will include all subjects who take at least one dose of study drug during the study. For the safety analysis set, subjects are assigned to a treatment group based on the treatment actually received, regardless the treatment randomized.

DATA HANDLING AND ELECTRONIC TRANSFER OF DATA

SAS data extracts will be transferred via sFTP (secure file transfer protocol) portal as per Medrio SOP on data transfer. Data files will be created as per the DMP (Data Management Plan).

ANALYSIS CONVENTIONS

Statistical Significance

Unless otherwise specified, statistical tests will be 2-sided for all analyses and the null hypothesis will be rejected at the significance level of $\alpha = 0.05$. P-values will be rounded to four decimal places before assessing statistical significance. In this Phase II study, no adjustment for multiple comparisons for the primary clinical, safety, and biochemical outcomes will be made. The multiplicity from testing secondary clinical, biochemical, and safety outcomes will be adjusted based on a Hochberg procedure (Sakamaki, 2013; Ye et al., 2012).

Missing Data

Although every effort will be made to minimize the amount of missing data, some level of missing data will be unavoidable for a variety of reasons. In general, data may be missing due to subject's early withdrawal from study. Patients discontinuing early from the study and reasons for withdrawal will be summarized by treatment group to see if any biases in the analyses may have been created by any differences between groups. For the study of time-to-event endpoints, patients completing the study and not experiencing an event of interest are considered censored to the event. The impact of missingness will be minimized in the analysis of the primary endpoint by performing the analysis using Kaplan-Meier estimate, where participants will be censored at their last assessed time point. The procedures outlined below describing what will be done when data are missing may be refined during the blind review of the data.

For this Phase II study, missing data imputation will only be done for the primary and secondary biochemical outcomes (i.e., plasma levels of citrulline, arginine, ornithine, and NOx). For the analysis of other safety and secondary endpoints, no imputation will be done for missing values.

Missing biochemical data, it will be minimized operationally as we are setting up significant oversight on sample logistics. Nonetheless, given the longitudinal nature of data collection for biochemical measures, there exist the possibility of data truncation due to patient death.

The analyses for the biochemical measures will be fitted with a mixed effects model (with random intercept and slope). The advantage of using a mixed effects model is the use of all available cases and only excluding patients with missing biochemical values at all data collection periods. The model will analyze patients who have values missing only at some assessment periods by allowing for the missing data using the within and between patient correlations. However, the mixed effects modeling approach may not necessarily reduce all the bias when the missing mechanism is informative or not-missing-at-random (NMAR). As a sensitivity analysis, we will apply an imputation approach based on pattern mixture modelling. Initially, we will assume an ignorable missingness mechanism and use multiple imputation to impute missing values. The pattern mixture modelling will be used to adjust the imputed values by a fixed value. Next, the mixed effects model will be fitted using the adjusted values for each imputed dataset and Rubin's rule will be used to combine the results.

While we have outlined potential strategies to handle missing data, the primary goal will be to mitigate the level of missingness utilizing the following steps: (1) the protocols and informed consent forms will clearly differentiate treatment discontinuation from study withdrawal; (2) site investigators will be trained about the importance of retention and steps to prevent missing data; (3) the consent forms will include a statement educating patients about the continued scientific importance of their data even if they discontinue study treatment early; (4) we will continue following patients after discharge via telehealth visits or other modalities (e.g., phone, texts, and emails); (5) we will take steps to be able to ascertain vital status in all randomized patients.

Analysis of 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 Chi-square test for categorical variables. Fisher's exact test will be used if ≥ 20% of the cells have expected cell count < 5. All statistical analyses will be done using SAS version 9.4 statistical software (SAS Institute Inc., Cary, NC).

Patient Disposition

Disposition tables including patients signed informed consent, randomized (safety analysis set, full analysis set, per protocol set, pharmacokinetics analysis set), drop out with reasons will be prepared.

The number of subjects will be tabulated overall for the following sets: safety population, subjects who completed study by period, and subjects who prematurely discontinued for study drug, for each treatment group and overall, as appropriate. Additionally, the number and percentage of subjects who discontinued study drug will be summarized by reason for each treatment group and overall. All reasons and primary reasons for discontinuation of study drug will be summarized as recorded on the eCRF by the following categories:

- Adverse event (AE)
- Protocol violation
- Lost to follow-up
- Withdrew by subject
- Lack of efficacy

Subjects may have more than one reason for discontinuing study drug, but they will be counted once for the total number of discontinuations. Subjects have only one primary reason for discontinuing study drug or discontinuing from the study.

Primary Endpoints

Primary Clinical Endpoint

- The primary clinical efficacy variable is time to intubation event in hours from the start of study infusion.

Statistical Method for the Primary Clinical Efficacy Endpoint

Kaplan-Meier estimates of the survival functions will be graphically displayed for each treatment group. Kaplan-Meier quartiles (25^{th} percentile and median) with two-sided 95% confidence intervals will be calculated if applicable. In addition, time to intubation will be summarized via displaying number of

subjects at risk, the percent of subjects that are censored, and Kaplan-Meier event rates with two-sided 95% confidence intervals.

A log-rank test stratified by the randomization stratification factor (age group) will be used to compare the time to intubation of the two treatment groups. The hazard ratio of IV Citrulline compared with placebo and its corresponding two-sided 95% confidence interval will be estimated using a Cox proportional hazards (PH) model with treatment groups as the independent variable and stratified by the age group.

Analysis of Covariates for Primary Clinical Endpoint

The covariates in Table 3 will be analyzed for the primary clinical outcome (time to intubation) in the ITT population. The 95% two-sided confidence interval of the hazard ratio of IV Citrulline compared with placebo, adjusted for each covariate separately from the Cox PH model will be obtained. Due to small sample size of this Phase II trial, the Cox PH model adjusted for all covariates simultaneously will not be performed. Additionally, each covariate will be re-examined and appropriately re-categorized if model convergence (i.e. quasi-separation) occurs due to sample size.

SECONDARY ENDPOINTS

Secondary Clinical Endpoints

- All-cause hospital mortality
- The requirement for intubation and invasive mechanical ventilation
- Difference in proportion of patients intubated
- Difference in proportion of patients intubated an on MV
- Length of ICU stay in hours
- Length of hospital stay in hours
- Length of oxygen therapy in hours
- Length of non-invasive mechanical ventilation in hours

Statistical Method for the Secondary Clinical Efficacy Endpoints

All-cause Hospital Mortality

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 Fisher's Exact Test. Additionally, time to death will also be analyzed using Kaplan-Meier estimates of the survival functions. A log-rank test will be used to compare time to death for the two treatment groups. The hazard ratio of IV Citrulline compared with placebo and its corresponding two-sided 95% confidence interval will be estimated using a Cox proportional hazards (PH) model with treatment groups as the independent variable.

Requirement for Intubation and Invasive Mechanical Ventilation

We will provide summary statistics (frequency and percentages) of the proportion of patients that require intubation and invasive mechanical ventilation by treatment groups. We will test the difference in proportion requiring intubation and invasive mechanical ventilation using Fisher's exact test.

Difference in Proportion of Patients Intubated

To compare the proportion of patients intubated across the two treatment groups, we will follow the same analytical plan for the requirement for intubation and invasive mechanical ventilation outcome.

Difference in Proportion of Patients Intubated and on MV

To compare the proportion of patients intubated across the two treatment groups, we will follow the same analytical plan for the requirement for intubation and invasive mechanical ventilation outcome.

Length of ICU Stay

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. We will consider ICU LOS a 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 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.

Length of Hospital Stay

Hospital length of stay will be defined as time from study enrollment to first hospital discharge. We will follow the same analytical plan for the length of ICU stay outcome.

Length of Oxygen Therapy in Hours

Length of Non-invasive Mechanical Ventilation

We will provide median and range of length of non-invasive mechanical ventilation separately for each treatment group. There is an expectation that some patients will not require mechanical ventilation; therefore, overall duration of mechanical ventilation will be modeled with a zero-inflated Poisson model using consent and post-infusion start time for two separate analysis.

Secondary Biochemical Endpoints

- Plasma levels of citrulline, Arginine and other amino acid intermediates will be evaluated at baseline, 2-hours, 12-hours, and study days 2, 4, 8, and 10. The PK-PD associations will be evaluated especially to explore associations with outcomes of interest as per clinical endpoints.
- Plasma levels of arginine at baseline, 2-hours, 12-hours, and study days 2, 4, 8, and 10.
- Plasma levels of ornithine at baseline, 2-hours, 12-hours, and study days 2, 4, 8, and 10.
- Plasma NOx levels at baseline, 2-hours, 12-hours, and study days 2, 4, 8, and 10.

Statistical Method for the Citrulline Biochemical Efficacy Endpoint

The analysis team will provide the means and standard deviations for citrulline plasma levels separately for each treatment group and for each assessment periods. 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). We will calculate the point estimates and their respective confidence intervals for the changes in patients' citrulline plasma levels for each intervention group and for the difference in the estimated change between intervention groups. Additionally, we will present the p-value of the difference in point estimates between intervention groups.

Statistical Method for the Biochemical Efficacy Endpoints (arginine, ornithine, and NOx)

For the secondary biochemical endpoint arginine, ornithine, and NOx, we will follow the same analytical method described to analyze plasma levels of citrulline.

Secondary Safety Endpoints

- Hemodynamic status (worst vasopressor dependency index through day 10)
- Number of reported adverse events

Statistical Method for the Safety Endpoint (Hemodynamic status)

The vasopressor dependency index (VDI) is a continuous variable calculated by dividing vasopressor index (summation of all pressors) by the mean arterial blood pressure (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. We will report descriptive statistics (mean ± SD) of baseline and worst VDI separately for each treatment group. Worst VDI will be compared between groups using an analysis of covariance (ANCOVA) with the baseline VDI as the covariate.

Statistical Method for the Secondary Safety Endpoint (Adverse events)

All reported adverse events will be tabulated and categorized by level of severity and reported by treatment groups. Adverse events will be evaluated further where causality is attributed as suspect.

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.