Statistical Analysis Plan (SAP)

Protocol Title:	A Randomized, Double-Blind, Placebo Controlled Study to Assess the Efficacy and Safety of Pulsed, Inhaled Nitric Oxide (iNO) Versus Placebo in Subjects With Mild or Moderate Coronavirus Disease (COVID-19)
Protocol Version No./Date:	Amendment 1.1 (19 August 2020)
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1.0 Approvals

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(NOTE: Electronic Signatures should only be used if all parties have the ability to eSign.)

2.0 Change History

Version/Date	Change Log
0.6	Draft Version
1.0 / 26-OCT-2020	Final Version

3.0 Table of Contents

4.0 Purpose

The Statistical Analysis Plan (SAP) describes the statistical methods to be used during the reporting and analyses of data collected under Bellerophon Pulse Technologies LLC Protocol PULSE-CVD19-001.

5.0 Scope

This SAP describes the statistical methods to be used during the reporting and analyses of data collected for Bellerophon Pulse Technologies LLC protocol PULSE-CVD19-001, titled "A Randomized, Double-Blind, Placebo Controlled Study to Assess the Efficacy and Safety of Pulsed, Inhaled Nitric Oxide (iNO) Versus Placebo in Subjects With Mild or Moderate Coronavirus Disease (COVID-19)".

The Statistical Analysis Plan outlines the following:

- Study Objectives
- Study Design
- Study Endpoints
- Applicable Study Definitions
- Statistical Methods

6.0 Introduction

The study protocol provides conduct for a randomized, double-blind, placebo-controlled study to assess the efficacy and safety of pulsed iNO compared to placebo in subjects with COVID-19 who are hospitalized and require supplemental oxygen without assisted ventilation. Subjects will receive placebo or iNO125 mcg/kg ideal body weight (IBW) /hr for 24 hours daily until resolution of hypoxemia, protocol defined respiratory failure, hospital discharge or Day 28, whichever occurs first. This study will be conducted with one interim analyses, when 100 subjects complete Day 28 on the protocol to assess safety and potential futility of the IP, as detailed in Section 8.0 of this SAP.

This SAP has been developed and should be read in conjunction with the study protocol and case report form (CRF) referenced on the signature page.

Changes made to the SAP after it has been approved but prior to final analyses of data will be documented in an amendment. Important changes to the SAP, along with the justification for the changes, will be described in the CSR. Changes to the protocol will require a SAP amendment ONLY if the changes are to a principal feature of the protocol.

7.0 Study Objectives

The study protocol objectives are as follows:

Primary Objective: To verify the efficacy of INOpulse in subjects with COVID-19.

Secondary Objective: To evaluate the safety of INOpulse in subjects with COVID-19.

8.0 Study Design

This is a randomized, double-blind, placebo-controlled study to assess the efficacy and safety of pulsed iNO compared to placebo in subjects with COVID-19 who are hospitalized and require supplemental oxygen without assisted ventilation. Subjects will receive placebo or iNO125 mcg/kg IBW/hr for 24 hours daily until resolution of hypoxemia, protocol defined respiratory failure, hospital discharge or Day 28, whichever occurs first. Subjects will be followed through 30 days after discontinuation of INOpulse therapy to assess their clinical status.

An unblinded independent Data Monitoring Committee (DMC) will review safety data including adverse events and adverse events of special interest. The roles and responsibilities of the DMC, including membership, scope, frequency of meetings and communication plan are defined in the current DMC charter on file with the sponsor. DMC members will evaluate the overall safety profile of INOpulse for subjects who received treatment compared to subjects who received the placebo.

There is one planned interim analyses for this study in order to assess potential futility of the INOpulse group versus placebo based on the primary endpoint of death/respiratory failure (DRF). The interim analysis will occur after the first 100 subjects complete 28 days on the protocol, and will evaluate interim safety and potential futility. The DMC will make the determination to either continue the trial, or to stop the trial due to safety concerns or futility, based on DRF. The interim analyses results will be evaluated by the DMC based on pre-specified criteria and stopping rules as detailed in this SAP. The final analysis will be conducted using the full sample size of all subjects enrolled, according to the population definitions ("Analysis Sets") described in Section 11.0 below.

8.1 Sample Size Considerations

Initial assessment of the sample size is based on the historical experience of ~30% of hospitalized COVID-19 subjects deteriorating to needing increased respiratory support and the preliminary experience in the Emergency Expanded Access that indicated ~15% of INOpulse-treated subjects deteriorate.

More recent data suggest that ~15% of hospitalized COVID-19 subjects deteriorate to needing increased respiratory support. We hypothesize that ~6% of iNO-treated subjects deteriorate.

Using 15% for the placebo arm and 6% for the INOpulse arm, a total sample size of 500 subjects yields 93% statistical power for a 1:1 randomized trial with a two-sided p-value of 0.05.

Based on this analysis of the DRF endpoint, we are targeting a sample size of 500 subjects.

8.2 Randomization and Stratification

Subjects will receive placebo or iNO125 mcg/kg IBW/hr for 24 hours daily until resolution of hypoxemia, protocol defined respiratory failure, hospital discharge or Day 28, whichever occurs first. Randomization will be stratified based on the following four [2-level] factors in Table 1 below:

Table 1: Stratification Factors

Factor	Low	High
Oxygen Use	≤5 L/min	>5 L/min
NT-proBNP	<320 pg/ml	≥320 pg/ml
# of Comorbidities	0 or 1	≥2
Remdesivir use	No	Yes

The four comorbidities considered are

1. Diabetes;
2. Hypertension;
3. Cardiovascular disease (including ischemic heart disease, heart failure, cerebrovascular disease, peripheral vascular disease);
4. Obesity (BMI ≥ 30).

9.0 Study Endpoints

Objectives	Endpoints
Primary	Primary endpoint
• The primary objective in this study is to verify the efficacy of INOpulse in subjects with COVID-19.	• Proportion of subjects who died or had respiratory failure (DRF as defined in Section 10.0 below) through Day 28.
	Secondary Endpoints

	• 8-point NIAID ordinal scale (Table 2 below) assessed at Day 7, 14, 28 and day of discharge.
	• Proportion of subjects to recover, defined as return to room air or baseline oxygen requirements, or discharged alive from hospital [Through Day 28].
	• Proportion of subjects discharged alive from hospital [Through Day 28]
	• Duration of hospitalization [Through Day 28].
	• Mortality [Through Day 28]:
	  o All-cause mortality
	  o Cardiopulmonary mortality
	• Proportion of subjects with a negative conversion of RT-PCR from a nasopharyngeal or bilateral nasal swab [through Day 28].

Secondary	
• The secondary objective in this study is to evaluate the safety of INOpulse in subjects with COVID-19.	• Proportion of subjects with adverse events leading to study drug discontinuation [Through Day 28].

Table 2: 8-Point NIAID Ordinal Scale

Score	Outcome
1	Death
2	Hospitalized, requiring mechanical ventilation or ECMO
3	Hospitalized, requiring non-invasive ventilation or high flow oxygen
4	Hospitalized, requiring supplemental oxygen
5	Hospitalized, not requiring supplemental oxygen - requiring ongoing medical care (COVID-19 related or otherwise)
6	Hospitalized, not requiring supplemental oxygen - not requiring ongoing medical care (COVID-19 related or otherwise)
7	Not hospitalized - limitation on activities and/or requiring home oxygen
8	Not hospitalized, no limitations on activities

10.0 Conventions and Derivations

The primary endpoint is the proportion of subjects who died or had respiratory failure (DRF) through day 28 (treatment day 27), where respiratory failure is defined as one of the following:

- Endotracheal intubation and mechanical ventilation;
- Extracorporeal membrane oxygenation;
- High-flow nasal cannula oxygen delivery (i.e., reinforced nasal cannula delivering heated, humidified oxygen with fraction of delivered oxygen ≥0.5 and flow rates of ≥30 l/min);
- Noninvasive positive pressure ventilation;
- Clinical diagnosis of respiratory failure with initiation of none of these measures only when clinical decision-making is driven solely by resource limitation or in the event the subject is not intubated due to do not intubate (DNI) or do not resuscitate (DNR) status.

Duration of therapy will be calculated as the date of first dose of study drug on the INOpulse Usage Form through the date of last dose of study drug on the End of Treatment CRF page.

Baseline is defined as the last assessment prior to the first dose of study drug.

Body Mass Index is defined as subject weight (kg) divided by height (m^2).

The age at COVID-19 diagnosis is defined as the number of years from the date of birth to the date of diagnosis of COVID-19, which will be derived based on the table below. No imputation will be done for missing disease diagnosis date (Year, Month, Day). The age calculation will be truncated to a whole number.

Table 3: Calculation for Age at COVID-19 Diagnosis

Observed Portion	Missing Portion	Formula to Calculate Duration
Year, Month, Day		(Date of Diagnosis – Date of Birth + 1)/365.25
Year, Month	Day	[Year (Date of Diagnosis) – Year (Date of Birth)] + [Month (Date of Diagnosis) – Month (Date of Birth)]/12
Year	Month, Day	[Year (Date of Diagnosis) – Year (Date of Birth)]

11.0 Analysis Sets

11.1 All Randomized Analysis Set

The All Randomized Analysis Set (ARAS) includes all subjects who are randomized in the study. That is the primary analysis set for by-subject listings.

11.2 Full Safety Set

The Full Safety Set (FSS) includes all randomized subjects who received any amount of study drug. This is the primary analysis set for safety analyses.

11.3 Full Efficacy Set

The Full Efficacy Set (FES) includes all randomized, COVID-confirmed subjects who received any amount of study drug. Note that subjects who began study treatment with high suspicion of COVID infection but subsequently had negative test results on the RT-PCR swab – will be excluded from FES.

11.4 Per Protocol Analysis Set

The Per-Protocol Analysis Set (PPAS) is a subset of FES, to be used for secondary analysis of efficacy. FES subjects with at least one of the following protocol deviations will be excluded from the PPAS:

- Subject did not meet all protocol-specified inclusion criteria or met at least one of the protocol-specified exclusion criteria.
- Subject received study drug other than the one that he/she was randomized to receive
- Subjects with any other protocol deviation identified in the course of study monitoring which, in the opinion of the Sponsor's Responsible Medical Officers based on blinded review, is likely to impact the validity of the data

12.0 Interim and Final Analyses

12.1 Interim Analysis

The interim analysis for futility is planned after ~100 subjects have completed assessments through Day 28 in order to compare the failure rate in the INOpulse group versus placebo for the primary endpoint of DRF (defined in Section 10.0 above). If the observed DRF rate in the INOpulse group is $\geq$ 2.5 percentage points higher than that of placebo ($\pi_t - \pi_c >= 0.025$), then the study will stop for futility (without regard to statistical significance).

12.2 Final Analysis

Final analyses outlined in this SAP will be carried out after:

- The SAP has been approved
- The study database has been locked and Bellerophon authorizes the release of the randomization assignments
- Protocol deviations have been categorized (into types of deviations), classified as important/ not important, and finalized
- Membership in the PPAS, based on blinded data and review, has been determined and finalized.

13.0 Statistical Methods

All analyses will use SAS version 9.4 or higher.

Data from all study sites will be pooled for analyses.

Unless otherwise noted, all summaries will be provided by treatment group: INOpulse and placebo. Demographic summaries will also be provided by stratification group (separately for each stratification factor, described in Section 8.2 above) as a subgroup analysis.

Unless otherwise noted, continuous variables will be summarized using number of observations (n), mean, standard deviation (std), median, first quartile (Q1), third quartile (Q3), minimum and maximum values. The minimum and maximum values will be displayed to the same level of precision as the raw data; the mean, median, Q1 and Q3 values to one additional decimal place and the std to two additional decimal places.

Categorical variables will be summarized using counts and percentages. Percentages will be rounded to one decimal place, except 100% will be displayed without any decimal places and percentages will not be displayed for zero counts.

All participants will be used in the analyses, as per the analysis populations described in Section 11.0 above, using all non-missing data available. Missing data in the logistic regression analysis of DFR will be analyzed as follows: If there is missing data for more than 5% of the cases for the outcome variable, a tipping point analysis will be performed. This will be done as a sensitivity analysis to determine whether or not the data is missing at random, and if the data is not missing at random, how robust is the parameter estimate in the model. The MI and MIANALYZE procedures in SAS[1] will be used to perform this analysis.

As a secondary sensitivity analysis, if there is missing data for more than 5% of the subjects at any time point in the NIAID assessments, the MI procedure in SAS will be utilized to impute missing values for NIAID score, and the statistical models will be run again using the imputed data points to compare the validity of the results using MIANALYZE.

1. SAS/STAT 14.2 User Guide. 2016, 6130 – 6135. SAS Institute Inc., Cary, NC.

Unless otherwise noted, values for missing laboratory and vital sign values will not be imputed. However, a missing baseline result will be replaced with a screening result, if available. If laboratory values for a subject are missing for any reason at a time point, the subject will be excluded from the calculation of summary statistics for that time point. If no pre-treatment laboratory value is available, the baseline value will be assumed to be normal (i.e., no grade [Grade 0]) for the summary of graded laboratory abnormalities.

For all tabulations of data by study time points according to a study day (e.g., Day 7, 14, 28, but not discharge or end of treatment), time windows of ±3 days will be used to associate data with a time point, as shown in Table 4 below.

Table 4: Visit Windows

Nominal Day / Visit	Window
Day 7	Days 4 – 10
Day 14	Days 11 – 17
Day 28	Days 25 – 31

If there are multiple records within the same window, the latest one will be used for analyses.

For analyses of laboratory data that are continuous in nature but are less than the lower limit of quantitation (LLOQ) or above the upper limit of quantitation will be imputed to the value of the lower or upper limit minus or plus 1 significant digit, respectively (e.g., if the result of a continuous laboratory test is < 30, a value of 29 will be assigned; if the result of a continuous laboratory test is < 30.0, a value of 29.9 will be assigned). If the results of continuous lab test is <1, the imputed value should be 0.9; If the results of the lab test is <0.1, the imputed value should be 0.09. The actual reported values will be provided in by-subject listings.

13.1 Subject Disposition

The number (and %) of subjects completing the study, and discontinuing from the study, will be tabulated by treatment group, along with reasons for discontinuation. The number of subjects screened (defined as providing informed consent) and who were screen failures, along with a summary of reasons for screen failure (i.e. eligibility criteria violations), will be provided. A by-subject listing of subjects who signed informed consent but were not randomized (screen failures) including their specific reason for exclusion will also be provided. Subjects who discontinued from the study will be summarized in a by-subject listing.

The number and percentage of subjects in each treatment group in the all randomized, full analysis, and per-protocol analysis sets will be provided.

A tabulation of the number and percentage of enrolled subjects at each center will be presented.

13.2 Demographic and Baseline Characteristics

Demographic and baseline characteristics (gender, race , ethnicity, age (years) , height (cm), weight (kg), and medical history will be summarized overall and by treatment group using descriptive statistics including sample size (n), mean, std, median, Q1, Q3, minimum, and maximum for continuous variables and numbers and percentages of subjects for categorical variables. This summary will be provided for the All Randomized analysis set and the FSS, FES and PPAS.

Demographics and medical history will be provided in by-subject listings.

13.3 Treatments

13.3.1 Extent of Study Drug Exposure

For each subject, the total number of hours of drug exposure will be calculated. The total duration of exposure to investigational product (in days) will be calculated as: Date of last dose – Date of first dose + 1. Hours and duration of drug exposure will be listed.

The first dose of treatment will be defined as Day 0, as stated in the Protocol.

13.3.2 Concomitant Medications

Prior and/ or concomitant medications will be coded according to WHODRUG (Version Global B3 2017SEP01 DDE+HD (Enhanced + Herbals)).

A medication or therapy, other than the study drug, is considered concomitant with study drug if administered on or after the administration of study drug. Prior medications or therapies are those administered prior to the administration of study drug.

Prior and/ or concomitant medications will be summarized separately by treatment group and the summary will include the number and percentage of subjects who received at least one medication and the number and percentage of subjects who received at least one medication by ATC classification level 3 and preferred term. Separate descriptive summaries will be provided by treatment group for the FES and PPAS.

All medications will be provided in a by-subject listing along with a column indicating whether the medication was prior or concomitant

Handling of missing or partial dates is defined in Appendix 2.

13.4 Important Protocol Deviations

A by-subject listing of randomized subjects with any eligibility criteria violations will be provided.

The number and percentage of randomized subjects with any important protocol deviation and the number and percentage of subjects with any important protocol deviation by deviation category will be summarized overall and by treatment group. A by-subject listing of all protocol deviations will be provided with columns identifying the date of the deviation, importance (important or not important), deviation category, and the standardized and verbatim descriptions of the deviation. All important protocol deviations will be summarized by using the FES.

13.5 Efficacy Analyses

The primary efficacy endpoint for this study is the proportion of subjects who died or had respiratory failure (DRF) through Day 28.

All subjects in the ARAS will be included in the analysis of the efficacy endpoints. The DRF proportion will also be calculated for the following subgroups: race (White Caucasian vs African American vs Others), gender, age group ($\leq$ 65 years vs > 65 years), and baseline disease severity (oxygen $\leq$5 vs >5 and comorbidities < 2 or $\geq$2).

13.5.1 Estimand

The main estimand is the odds ratio for the likelihood of DRF, comparing the INOpulse group to the placebo group.

The main estimand is defined by the following attributes:

(1) Treatment regimens for patients to be evaluated:

INOpulse at 125 micrograms/kg IBW/hr versus INOpulse placebo

(2) Populations of patients targeted by the clinical question:

The patients targeted in this analysis are all randomized patients, grouped according to randomized treatment assignment.

(3) Patient-level outcome to be measured:

The outcome being measured is the likelihood of death or respiratory failure at Day 28.

(4) Intercurrent events:

The analysis will be done regardless of the use of rescue medications, protocol violations, or investigational product discontinuation. Intercurrent events which are protocol deviations will be summarized in table format. The outcome of DRF is binary, with the event being DFR, and the non-event being any other outcome. Therefore, other modifications due to intercurrent events, such as death from another cause or a subject receiving another therapy, will not be made to the outcome variable. Death will not be considered an intercurrent event for the primary endpoint as it is part of that endpoint definition. Subjects will be analyzed using an "intent to treat" approach. Sensitivity analysis of the primary outcome using the PPAS set will be conducted to evaluate the robustness of the parameter estimate.

(5) Population-level summary measure

The population-level summary is the estimated likelihood of DFR, adjusted for treatment, baseline oxygen level (as a continuous covariate), number of co-morbidities (as a discrete numeric covariate), and age (as a continuous covariate).

13.5.2 Type I Error Rate

A gatekeeping methodology will be utilized for the primary and secondary hypothesis testing.[2] This gatekeeping method controls the study-wise Type I error rate at 5% associated with the study's primary and secondary endpoint families. If the primary endpoint is found to be statistically significant, the p-value of 5% will be used to test the first of the secondary endpoints. This testing of the secondary endpoints will continue in a hierarchical manner, until one of the statistical tests is not found to be significant. If this occurs, none of the remaining secondary endpoints will be tested. The hierarchy for the secondary endpoints is shown as they are listed in Section 13.5.5. If the primary endpoint is found not to be statistically significant, then the statistical testing of the secondary endpoint family will not be performed.

2. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/multiple-endpoints-clinical-trials-guidance-industry

13.5.3 Hypothesis Testing

Primary Endpoint (DRF): The statistical comparison of the proportion of subjects who experience respiratory failure (as defined in Section 10.0 above) or death through day 28 will be performed. Based on recent clinical experience, the overall proportion of hospitalized COVID19 patients who experience respiratory failure or death is about 15%. It is hypothesized that the INOpulse therapy will reduce the respiratory failure rate to 6%. Using a logistic regression analysis, this corresponds to an odds ratio of 0.36, meaning a 64% reduction in the likelihood of DFR for those patients in the INOpulse group. Formally, this hypotheses can be stated as follows:

$$H_o: \beta_1 = 0$$

$$H_1: \beta_1 = -1.017$$

where the estimand β_1 is the coefficient in the logistic regression model for treatment effect. A sample size of 500 patients yields a 93% statistical power to test this hypothesis, with a two-sided Type 1 error rate of

5%, for a logistic regression model with a binary covariate[3]. Conservatively, this estimand will be adjusted for age, baseline oxygen level, number of co-morbidities, and treatment, as described in Section 13.5.4 below.

3 F.Hsieh, D.Bloch and M.Larsen. *A Simple Method of Sample Size Calculation for Linear and Logistic Regression.* Statistics in Medicine, 17, 1623-1634. Stanford, 1998.

13.5.4 Statistical Modeling of Primary Estimand

A logistic regression model will be utilized (which will allow us to adjust for covariates) to model the outcome of the proportion of subjects who experience respiratory failure or death through day 28. The outcome variable of respiratory failure (response) will be binary (yes/no). Factors in the model will be: treatment, baseline oxygen level (as a continuous covariate), number of co-morbidities (as a discrete numeric covariate), and age (as a continuous covariate). The estimand of the effect of treatment on the likelihood of respiratory failure will be calculated as an odds ratios with a 95% confidence interval, adjusted for age, baseline oxygen level, and number of co-morbidities. A difference in proportions will also be provided as part of the overall analysis along with a 95% confidence interval (Refer Appendix 4).

Example SAS code for the logistic regression model is as follows:

```
proc logistic;
class treatment no_comorbidities;
model response = age baseline_oxygen_level no_comorbidities treatment;
run;
```

Baseline NIAID score will not be adjusted for, as all patients in this trial will have ordinal scale score of 4 (hospitalized requiring oxygen) at the time of trial entry, and the baseline disease severity will already be captured to a large extent by the oxygen level requirements.

As a sensitivity analysis, the primary analysis of DRF described in Section 12 above will be repeated on the PPAS.

13.5.5 Secondary Endpoints

Secondary efficacy analyses will be performed on the secondary endpoints using the subjects in the ARAS.

Summary measures of the secondary endpoints (i.e. totals, means, proportions) will also be calculated for the following subgroups: race, gender, age group (≤ 65 years vs > 65 years), and baseline disease severity (oxygen ≤5 vs >5 and comorbidities < 2 or ≥2).

13.5.5.1 NIAID Score

Clinical status using an 8-point NIAID ordinal scale (Table 2 above) will be assessed on days 7, 14, 28 and Day of Discharge. Descriptive statistics (totals and percentages) will be summarized for subjects overall, and by treatment group and day of assessment. The effect of treatment on NIAID score over time will be evaluated using a mixed effects model with repeated measures (MMRM). The estimand will be the slope of the regression coefficient for the treatment effect, adjusted for covariates as shown in the example SAS code below:

```
proc mixed method=ml covtest;
class patient treatment no_comorbidities;
model NIAID_Score = age baseline_oxygen_level no_comorbidities treatment / s;
repeated / type=un subject=patient r;
run;
```

The significance of model coefficients will be evaluated using F tests.

13.5.5.2 Subject Recovery

The proportion of subjects to recover, defined as return to room air or baseline oxygen requirements, or discharged alive from the hospital, through day 28, will be summarized using descriptive statistics (totals and percentages) for subjects overall and by treatment group. The estimand of the proportion of recovery/discharge will be calculated by treatment group, along with 95% confidence intervals. A chi-square test will be used to test the significance in the proportion of subjects who recovered between the INOpulse and placebo treatment groups.

13.5.5.3 Subject Hospital Discharge

The proportion of subjects to be discharged alive from the hospital, through day 28, will be summarized using descriptive statistics (totals and percentages) for subjects overall and by treatment group. The estimand of the proportion of hospital discharge will be calculated by treatment group, along with 95% confidence intervals. A chi-square test will be used to test the significance in the proportion of subjects who are discharged between the INOpulse and placebo treatment groups.

13.5.5.4 Subject Hospital Duration

Subject hospital duration will be defined as the time from Day 1 (start of study treatment) to the day of hospital discharge or Day 28 of treatment for patients who are still hospitalized. If a patient has expired on or before Day 27 of treatment, they will be assigned a value of 28 days. Duration of hospital stay will be summarized (n, mean, std, median, Q1, Q3, minimum, and maximum) overall and by treatment group. The estimand of the median hospital duration will be summarized by treatment group, along with 95% confidence intervals. The non-parametric Mann-Whitney test will be used to test the difference in the duration of hospital stay between the two treatment groups.

13.5.5.5 Mortality

All-cause mortality will be defined as death from any cause through day 28 (treatment day 27). The all-cause mortality will be summarized using proportions, with all subjects in the ARAS included in the denominator.

Disease-specific mortality will be defined as death due to cardiopulmonary causes through day 28 (treatment day 27). This will be defined as having an adverse event under the respiratory body system/SOC, with outcome of death. It will be summarized using proportions, but the denominator will be the FES, excluding [subjects with] other causes of death through day 28.

Mortality rates will be displayed overall and by treatment group, age group, and gender as a subgroup analysis. The estimand of the proportion of mortality will be calculated by treatment group, along with 95% confidence intervals. A chi-square test will be used to test the significance in the proportion of mortality between the iNO and placebo treatment groups.

13.5.5.6 RT-PCR Conversion

Confirmation of COVID-19 diagnosis will be done through RT-PCR testing. Samples will be taken at baseline and End of Treatment/discharge. RT-PCR results will be summarized as positive or negative using proportions, overall and by treatment group; this will be done in the ARAS dataset, which by definition requires positive at baseline. Among subjects who had a positive RT-PCR test at study enrollment, the test positivity rate at hospital discharge will be calculated by treatment group, along with 95% confidence intervals. A chi-square test will be used to test the significance in the proportion of positivity between the INOpulse and placebo treatment groups at discharge. Death will be imputed as a positive conversion even in the case the subject was a negative conversion and died.

13.6 Safety Analyses

The primary safety endpoint is the proportion of subjects who experience adverse events (through Day 28) leading to study discontinuation. This proportion will be displayed overall for all subjects, and by treatment group

13.6.1 Data Monitoring Committee Review

This study will utilize an independent DMC to monitor and review data periodically throughout the trial, at a minimum monthly, as described in the current DMC charter on file with the sponsor. Adverse events will be summarized in tables for all patients and by treatment group. Patient listings will also be provided. The analysis will include all patients in the ARAS, and the data will be unblinded to the DMC. Treatment futility will be determined after 100 patients have been on-study for up to 28 days. The stopping rule criteria for futility using the interim analysis data are described in Section 12.1 of this SAP.

13.6.2 Adverse Events

Treatment-Emergent Adverse Events (TEAEs) are defined as any AEs with an onset date on or after the date of the first dose of IP or any worsening of a preexisting medical condition/AE with onset after the start of IP and throughout the study. An AE leading to treatment discontinuation is defined as an AE where action taken on study drug is Drug Withdrawn. AEs will be coded using the Medical Dictionary for Regulatory Activities MedDRA version 23.0.

An overall summary of TEAEs, including the number of events reported, the number and percentage of participants reporting at least one TEAE, the number and percentage of participants discontinuing due to an adverse event, the number and percentage of participants with at least one serious adverse event, and the number and percentage of deaths will be presented.

The most frequent adverse events (>=5%) will be compared between the INOpulse and placebo groups using a forest plot. The forest plot will display odds ratios of the incidence rate for each AE along with 95% confidence intervals.

A table of all anticipated and unanticipated deaths due to any cause, with number and frequency of such events in each arm/group of the clinical study, as well as the number of occurrences for each event. This also requires notification if the death is causally related to treatment for each reporting group.

A breakdown of the number and percentage of participants reporting each adverse event, categorized by System Organ Class (SOC) and preferred term (PT), will be presented. Note that counting will be by participant not event and participants are only counted once within each SOC and PT.

A further tabulation of these data, categorized by relationship to INOpulse, will be presented. Participants with multiple events within a particular body system or preferred term will be counted under the category of their drug-related event (related and not related) within SOC and PT. Relationship to INOpulse is categorized as related (rated “possible” or “related” in the CRF) or not related (rated “unlikely” or “not related” in the CRF).

A summary of events reported, categorized by severity, will also be provided. Participants with multiple events within a particular SOC and PT will be counted under the category of their most severe event in the following order: severe> moderate> mild within that SOC and PT.

A summary of adverse events leading to discontinuation of INOpulse will be provided, grouped by body system and preferred term.

The following events will be considered AEs of special interest (AESI). In addition, AESIs will also include medical device deficiencies and/or complaints.

- All respiratory AEs
- Incidence of methemoglobin (MetHb) levels ≥ 7.0% in blood

- New symptoms that may be associated with local nasal irritation: nasal erythema, epistaxis and/or coryza
- New or worsening symptoms of left heart failure or pulmonary edema
- Any decrease in systemic oxygenation measured by either PaO2 or oxygen saturation by pulse oximeter (SpO2) deemed by the Investigator to be clinically significant
- New symptoms that may be due to rebound effects associated with a temporal acute withdrawal of study drug (i.e., symptoms occurring within 20 minutes of acute withdrawal and including those associated with device malfunction or failure): hypoxemia, bradycardia, tachycardia, systemic hypotension, syncope, near-syncope, ventricular fibrillation and/or cardiac arrest.

All adverse events (including non-treatment-emergent events) recorded on the CRF will be listed.

Handling of missing or partial AE start dates is defined in Appendix 2.

13.6.3 Deaths and Serious Adverse Events

The number and percentage of subjects reporting a SAE, by SOC and PT will be summarized by treatment group.

All deaths and SAEs will be presented in a by-subject level listing.

13.6.4 Laboratory Data

All laboratory parameters collected, along with change from baseline, will be summarized (n, mean, std, median, Q1, Q3, min, max) by treatment group and study time point. The lab parameters will include:

- Hematology - CBC
- Chemistry
  - Sodium
  - Potassium
  - Chloride
  - CO2
  - BUN
  - Creatinine
  - Glucose
- Liver Function Tests (LFTs)
  - AST
  - ALT
  - Total Bilirubin
- Lipid/Protein Panel
  - Triglycerides
  - High Sensitivity Cardiac Troponin (HS-cTN)
  - Procalcitonin
  - Ferritin
  - Fibrinogen
  - D-dimer
  - NT-proBNP

  - LDH
- Factors/Other Proteins
  - CRP
  - Von Willebrand's Factor
  - Factor VIII Activity
  - Protein C
  - Protein S
  - Homocysteine
  - Antithrombin 3
  - Thrombelastography (TEG)
  - G6PD deficiency
  - Prothrombin gene G2021A

Laboratory parameters will be grouped this way in the summary tables. Abnormal values for selected parameters will be graded by the central laboratory using criteria based on CTCAE Version 5.0 (SAP Appendix 3).

13.6.5 Vital Signs

Values for heart rate (HR), respiratory rate (RR), blood pressure (BP, systolic and diastolic), and temperature, and oxygen flow rate will be summarized (n, mean, std, median, Q1, Q3, minimum, and maximum) by treatment group and study time-point along with the change from baseline for each parameter at each scheduled post-baseline assessment.

All vital sign values for all subjects will also be provided in by-subject listings.

13.6.6 Other Observations Related to Safety

By-subject listings for physical examination, methemoglobin and 12-lead ECG findings will be provided.

14.0 References

1. SAS/STAT 14.2 User Guide. 2016, 6130 – 6135. SAS Institute Inc., Cary, NC.
2. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/multiple-endpoints-clinical-trials-guidance-industry
3. F.Hsieh, D.Bloch and M.Larsen. *A Simple Method of Sample Size Calculation for Linear and Logistic Regression.* Statistics in Medicine, 17, 1623-1634. Stanford, 1998.

Appendix 1: Glossary of Abbreviations

Abbreviation	Full Term
AE	Adverse event
AESI	Adverse Events of Special Interest
ALT	Alanine Aminotransferase
ARAS	All randomized analysis set
AST	Aspartate Aminotransferase
ATC	Anatomic Therapeutic Classification
CBC	Complete Blood Count
CO2	Carbon Dioxide
CP	Conditional Power
CRF	Case Report Form
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DRF	Death or Respiratory Failure
ECG	Electrocardiogram
FES	Full Efficacy Set
FSS	Full Safety Set
HR	Hazard Ratio
IBW	Ideal Body Weight
iNO	Inhaled Nitric Oxide
IP	Investigational Product
ITT	Intention-to-treat
LFT	Liver Function Test
LDH	Lactate Dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MetHb	Methemoglobin
NIAID	National Institute of Allergy and Infectious Diseases
PaO2	Partial Pressure of Oxygen
PPAS	Per Protocol Analysis Set
PT	Preferred Term
RT-PCR	Reverse transcription polymerase chain reaction
SAP	Statistical Analysis Plan

SAE	Serious Adverse Event
SOC	System Organ Class
SpO2	Oxygen Saturation
TEAE	Treatment Emergent Adverse Event
TEG	Thrombelastography

Appendix 2: Imputation for Partial or Missing Dates

Partial or missing dates will only be imputed for AEs and concomitant medications, not for any other dates. If dates are missing or incomplete for an AE (including deaths) or concomitant medication, the following algorithm will be used for imputation:

Table 5: Imputation Rules for Partial or Missing Start Dates

Start Date		Stop Date: Complete: *yyyymmdd* <1st dose	Stop Date: Complete: *yyyymmdd* ≥1st dose	Stop Date: Partial: *yyyymm* <1st dose *yyyymm*	Stop Date: Partial: *yyyymm* ≥1st dose *yyyymm*	Stop Date: Partial: *yyyy* <1st dose *yyyy*	Stop Date: Partial: *yyyy* ≥1st dose *yyyy*	Stop Date: missing
Partial: *yyyymm*	= 1st dose *yyyymm*	2	1	n/a	1	n/a	1	1
	≠ 1st dose *yyyymm*		2	2	2	2	2	2
Partial: *yyyy*	= 1st dose *yyyy*	3	1	3	1	n/a	1	1
	≠ 1st dose *yyyy*		3		3	3	3	3
Missing		4	1	4	1	4	1	1

1 = Impute the date of first dose
2 = Impute the first of the month
3 = Impute January 1 of the year
4 = Impute January 1 of the stop year
Note: If the start date imputation leads to a start date that is after the stop date, then do not impute the start date.

Imputation rules for partial or missing stop dates:

1. Initial imputation
    a. For partial stop date "mmyyyy", impute the last of the month.
    b. For partial stop date "yyyy", impute December 31 of the year.
    c. For completely missing stop date, do not impute.
2. If the stop date imputation leads to a stop date that is after the death date, then impute the stop date as the death date.
3. If the stop date imputation leads to a stop date that is before the start date, then there is a data error and do not impute the stop date (i.e. set the stop date as missing).

Imputation rules for partial or missing death dates:

1. If death year and month are available but day is missing:
    a. If "mmyyyy" for last contact date = "mmyyyy" for death date, set death date to the day after the last contact date.
    b. If "mmyyyy" for last contact date < "mmyyyy" for death date, set death date to the first day of the death month.
    c. If "mmyyyy" for last contact date > "mmyyyy" for death date, data error and do not impute.
2. If both month and day are missing for death date or a death date is totally missing, set death date to the day after the last contact date.

The imputed dates will be used to assess whether AEs should be considered as treatment-emergent and if concomitant medications should be included in the safety summaries, however the original, partial dates will be included in data listings.

Appendix 3: Abnormal Values for Selected Laboratory Parameters

Abnormal values for selected parameters will be graded by the central laboratory using criteria based on CTCAE Version 5.0.

Appendix 4: Tables, Figures, and Listings

Table Number	Title	Population
Table 14.1.1.1	Subject Analysis Sets	All Randomized Analysis Set
Table 14.1.1.2	Enrollment by Center	All Enrolled Subjects
Table 14.1.1.3	Subject Disposition	Full Efficacy Set
Table 14.1.1.5.1	Important Protocol Deviations	All Randomized Analysis Set
Table 14.1.1.5.2	Important Protocol Deviations	Full Efficacy Set
Table 14.1.2.1	Demographic Characteristics	All Randomized Analysis Set
Table 14.1.2.2	Demographic Characteristics	Full Safety Set
Table 14.1.2.3	Demographic Characteristics	Full Efficacy Set
Table 14.1.2.4	Demographic Characteristics	Per Protocol Analysis Set
Table 14.1.3.1	Medical History by System Organ Class and Preferred Term	All Randomized Analysis Set
Table 14.1.3.2	Medical History by System Organ Class and Preferred Term	Full Safety Set
Table 14.1.3.3	Medical History by System Organ Class and Preferred Term	Full Efficacy Set
Table 14.1.3.4	Medical History by System Organ Class and Preferred Term	Per Protocol Analysis Set
Table 14.1.4.1	Prior Medications by ATC Classification and WHO Drug Dictionary Preferred Term	Full Efficacy Set
Table 14.1.4.2	Prior Medications by WHO Drug Dictionary Preferred Term	Per Protocol Analysis Set
Table 14.1.5.1	Concomitant Medications by WHO Drug Dictionary Preferred Term	Full Efficacy Set
Table 14.1.5.2	Concomitant Medications by WHO Drug Dictionary Preferred Term	Per Protocol Analysis Set
Table 14.2.1.1	Analysis of Patients who Died or had Respiratory Failure	All Randomized Analysis Set
Table 14.2.1.1.1	Analysis of Patients who Died or had Respiratory Failure by Race	All Randomized Analysis Set
Table 14.2.1.1.2	Analysis of Patients who Died or had Respiratory Failure by Gender	All Randomized Analysis Set
Table 14.2.1.1.3	Analysis of Patients who Died or had Respiratory Failure by Age Group	All Randomized Analysis Set
Table 14.2.1.1.4	Analysis of Patients who Died or had Respiratory Failure by Baseline Oxygen Level	All Randomized Analysis Set
Table 14.2.1.1.5	Analysis of Patients who Died or had Respiratory Failure by No. of Co-Morbidities	All Randomized Analysis Set
Table 14.2.1.2	Analysis of Patients who Died or had Respiratory Failure	Per Protocol Analysis Set
Table 14.2.1.3	Logistic Regression Analysis of Patients who Died or had Respiratory Failure (DFR)	All Randomized Analysis Set
Table 14.2.1.4	Logistic Regression Analysis of Patients who Died or had Respiratory Failure (DFR)	Per Protocol Analysis Set
Table 14.2.1.5	Tipping Point Analysis of Logistic Regression Model	All Randomized Analysis Set

Table Number	Title	Population
Table 14.2.1.6	Tipping Point Analysis of Logistic Regression Model	Per Protocol Analysis Set
Table 14.2.2.1	Summary of NIAID Score by Time Point	All Randomized Analysis Set
Table 14.2.2.1.1	Summary of NIAID Score by Time Point Within Race	All Randomized Analysis Set
Table 14.2.2.1.2	Summary of NIAID Score by Time Point Within Gender	All Randomized Analysis Set
Table 14.2.2.1.3	Summary of NIAID Score by Time Point Within Age Group	All Randomized Analysis Set
Table 14.2.2.1.4	Summary of NIAID Score by Time Point Within Baseline Oxygen Level	All Randomized Analysis Set
Table 14.2.2.1.5	Summary of NIAID Score by Time Point Within No. of Co-Morbidities	All Randomized Analysis Set
Table 14.2.2.2	Mixed Effects Repeated Measures Model of NIAID Score	All Randomized Analysis Set
Table 14.2.2.3	Summary of Subject Recovery	All Randomized Analysis Set
Table 14.2.2.3.1	Summary of Subject Recovery by Race	All Randomized Analysis Set
Table 14.2.2.3.2	Summary of Subject Recovery by Gender	All Randomized Analysis Set
Table 14.2.2.3.3	Summary of Subject Recovery by Age Group	All Randomized Analysis Set
Table 14.2.2.3.4	Summary of Subject Recovery by Baseline Oxygen Level	All Randomized Analysis Set
Table 14.2.2.3.5	Summary of Subject Recovery by No. of Co-Morbidities	All Randomized Analysis Set
Table 14.2.2.4	Summary of Subject Hospital Discharge	All Randomized Analysis Set
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Table 14.2.2.4.2	Summary of Subject Hospital Discharge by Gender	All Randomized Analysis Set
Table 14.2.2.4.3	Summary of Subject Hospital Discharge by Age Group	All Randomized Analysis Set
Table 14.2.2.4.4	Summary of Subject Hospital Discharge by Baseline Oxygen Level	All Randomized Analysis Set
Table 14.2.2.4.5	Summary of Subject Hospital Discharge by No. of Co-Morbidities	All Randomized Analysis Set
Table 14.2.2.5	Summary and Analysis of Hospital Duration	All Randomized Analysis Set
Table 14.2.2.5.1	Summary and Analysis of Hospital Duration by Race	All Randomized Analysis Set
Table 14.2.2.5.2	Summary and Analysis of Hospital Duration by Gender	All Randomized Analysis Set
Table 14.2.2.5.3	Summary and Analysis of Hospital Duration by Age Group	All Randomized Analysis Set
Table 14.2.2.5.4	Summary and Analysis of Hospital Duration by Baseline Oxygen Level	All Randomized Analysis Set
Table 14.2.2.5.5	Summary and Analysis of Hospital Duration by No. of Co-Morbidities	All Randomized Analysis Set
Table 14.2.2.6	All-Cause Mortality	All Randomized Analysis Set
Table 14.2.2.6.1	All-Cause Mortality by Race	All Randomized Analysis Set
Table 14.2.2.6.2	All-Cause Mortality by Gender	All Randomized Analysis Set
Table 14.2.2.6.3	All-Cause Mortality by Age Group	All Randomized Analysis Set
Table 14.2.2.6.4	All-Cause Mortality by Baseline Oxygen Level	All Randomized Analysis Set
Table 14.2.2.6.5	All-Cause Mortality by No. of Co-Morbidities	All Randomized Analysis Set

Table Number	Title	Population
Table 14.2.2.7	Disease-Specific Mortality	All Randomized Analysis Set
Table 14.2.2.7.1	Disease-Specific Mortality by Race	All Randomized Analysis Set
Table 14.2.2.7.2	Disease-Specific Mortality by Gender	All Randomized Analysis Set
Table 14.2.2.7.3	Disease-Specific Mortality by Age Group	All Randomized Analysis Set
Table 14.2.2.7.4	Disease-Specific Mortality by Baseline Oxygen Level	All Randomized Analysis Set
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Table 14.2.2.8	Summary of RT-PCR Results by Time Point	All Randomized Analysis Set
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Table 14.2.2.8.2	Summary of RT-PCR Results by Time Point by Gender	All Randomized Analysis Set
Table 14.2.2.8.3	Summary of RT-PCR Results by Time Point by Age Group	All Randomized Analysis Set
Table 14.2.2.8.4	Summary of RT-PCR Results by Time Point by Baseline Oxygen Level	All Randomized Analysis Set
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Table 14.3.2.1	Overall Summary of Treatment-Emergent Adverse Events	Full Safety Set
Table 14.3.2.2	Treatment-Emergent Adverse Events by System Organ Class and Preferred Term	Full Safety Set
Table 14.3.2.3	Serious Treatment-Emergent Adverse Events by System Organ Class and Preferred Term	Full Safety Set
Table 14.3.2.4	Treatment-Emergent Adverse Events Leading to Discontinuation of IP by System Organ Class and Preferred Term	Full Safety Set
Table 14.3.2.5	Treatment-Emergent Adverse Events with Outcome of Death by System Organ Class and Preferred Term	Full Safety Set
Table 14.3.2.6	Treatment-Emergent Adverse Events by System Organ Class, Preferred Term, and Relationship to IP	Full Safety Set
Table 14.3.2.7	Treatment-Emergent Adverse Events by System Organ Class, Preferred Term, and Maximum Severity	Full Safety Set
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Table 14.3.3.1.3	Biochemistry Results by Time Point – Liver Function Tests	Full Safety Set
Table 14.3.3.1.4	Biochemistry Results by Time Point – Lipid/Protein Panel	Full Safety Set
Table 14.3.3.1.5	Biochemistry Results by Time Point – Factors/Other Proteins	Full Safety Set

Table Number	Title	Population
Table 14.3.4.1	Vital Sign Results by Time Point	Full Safety Set
Table 14.3.5.1	Electrocardiogram Results	Full Safety Set
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Figure 14.2.1.1	Forest Plot of Frequent Adverse Events	Full Safety Set
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Listing 16.2.1.1	Discontinued Subjects	All Randomized Analysis Set
Listing 16.2.1.2	Discontinuation of Investigational Product (IP)	All Randomized Analysis Set
Listing 16.2.2	Protocol Deviations	All Randomized Analysis Set
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Listing 16.2.4.1	Demographic and Baseline Data	All Randomized Analysis Set
Listing 16.2.4.2	General Medical History	All Randomized Analysis Set
Listing 16.2.4.3	Prior and Concomitant Medications	All Randomized Analysis Set
Listing 16.2.5.1	Administration of Investigational Product – INO Pulse Usage	All Randomized Analysis Set
Listing 16.2.5.3	Treatment Compliance	All Randomized Analysis Set
Listing 16.2.6.1	NIAID Score Results	All Randomized Analysis Set
Listing 16.2.7.1	Treatment-Emergent Adverse Events	All Randomized Analysis Set
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Listing 16.2.7.3	Death Details	All Randomized Analysis Set
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Listing 16.2.8.5	Pregnancy Test Results (Females of Childbearing Potential only)	Full Safety Set
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Listing 16.2.11	X-Ray Results	Full Safety Set
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Listing 16.2.14	Physical Examinations	Full Safety Set