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Randomized, double-blind, placebo-controlled, pharmacokinetic, pharmacodynamic study of Trans Sodium Crocetinate utilizing Transcutaneous Oximetry Measurement in healthy volunteers

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STATISTICAL ANALYSIS PLAN

For:

Diffusion Pharmaceuticals Inc.

PROTOCOL No. 200-301

Randomized, Double-blind, Placebo-controlled, Pharmacokinetic, Pharmacodynamic Study of Trans Sodium Crocetinate utilizing Transcutaneous Oximetry Measurement in Healthy Volunteers

Altasciences Project No. DIF-P3-945

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ALTASCIENCES

STATISTICAL ANALYSIS PLAN APPROVAL

We have carefully read this statistical analysis plan and agree it contains the necessary information required to handle the statistical analysis of study data.

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8 Apr 2021
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VERSION CONTROL

Version	Date	Author	Description of Changes
1.0	2021/04/08		

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ABBREVIATIONS

AE	Adverse event
ATC	Anatomical therapeutic chemical
AUEC	Area under effect time curve
BMI	Body mass index
BMP	Basic metabolic panel
CBC	Complete blood count
CI	Confidence interval
CRF	Case report form
CSR	Clinical study report
CV%	Coefficient of variability
DMP`	Data management plan
DTS	Deviation tracking system
ECG	Electrocardiogram
EOS	End of study
ET	Early termination
ICF	Informed consent form
ICH	International Conference on Harmonisation
ITT	Intent-to-treat
IV	Intravenous
LLOQ	Lower limit of quantitation
ln	Natural log
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
NCA	Non-compartmental analysis
O_2	Oxygen
PD	Pharmacodynamics(s)
PK	Pharmacokinetic(s)
PP	Per protocol
PT	Preferred term
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SOC	System organ class
SOP	Standard operating procedure
SpO_2	Oxygen saturation
TCOM	Transcutaneous oximetry
$tcpO_2$	Transcutaneous tissue oxygen tension

TEAE	Treatment-emergent adverse event
TFLs	Tables, figures, and listings
TSC	Trans Sodium Crocetinate
WHO-DDE	World Health Organization Drug Dictionary- Enhanced

1 INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to prospectively outline the types of analyses and presentations of the data that will form the basis for conclusions regarding this clinical investigation. The analyses defined in this plan should answer the safety, pharmacokinetic (PK), and pharmacodynamics (PD) objectives outlined in the protocol, and explain in detail how the data will be handled and analyzed, adhering to commonly accepted standards and practices for biostatistical analysis in the medical device industry.

This document contains information to support the generation of a Clinical Study Report (CSR) for Clinical Protocol 200-301, including detailed descriptions of the statistical methods to be applied, as well as the analysis summary tables and figures and subject data listings intended to present the analysis results.

This clinical trial is designed to further clarify and optimize the dosing of trans sodium crocetinate (TSC) by directly measuring tcpO2 in multiple locations on the lower extremity of a healthy volunteer after a single dose of TSC, and determine if subjecting the subjects to 100% O2 or Room Air (RA) is a more sensitive for evaluating the effect of TSC.

The sample size selected for this clinical investigation with 6 dose groups is based on clinical judgement. While hypotheses will be evaluated at the end of the study, there is no pre-specified statistical power that was used to establish the sample size.

The planned analyses identified in this SAP may be included in regulatory submissions, medical presentations and manuscripts. Exploratory analyses, not identified in this SAP, may be performed to support the clinical development program. Any post-hoc or unplanned analyses that are performed but not identified in this SAP will be clearly identified in the CSR. The structure and content of this SAP provides sufficient detail to meet the requirements identified by the Food and Drug Administration (FDA) and the International Conference on Harmonization (ICH) Guidance on Statistical Principles for Clinical Trials.

The analyses described in the SAP are based upon the final protocol version Amendment 2: dated 2021/03/09.

2 STUDY OBJECTIVES

The objectives of the study and corresponding study endpoints are detailed in Table 1.

Table 1: Objectives and related endpoints

Objective	Endpoint	Analysis
Primary		
To determine the dose-response of TSC on $tcpO_2$ following a single administration of TSC in subjects breathing O_2.	The primary PD endpoints are: • Time-matched change in $tcpO_2$ levels • The following PD parameters will be calculated for time-matched change in $tcpO_2$: ○ AUEC, E_{max}, and TE_{max}	Refer to Section 7
Secondary		
To evaluate the safety and the tolerability of TSC on $tcpO_2$ following a single administration of TSC in subjects breathing O_2.	Assessment of adverse events, new medications, laboratory (CBC, BMP), vital signs, and SpO_2.	Refer to Section 8

Abbreviations: BMP = basic metabolic panel; CBC = complete blood count; O_2 = oxygen; PD = pharmacodynamic; SpO_2 = oxygen saturation; $tcpO_2$ = transcutaneous tissue oxygen tension; TSC = trans sodium crocetinate

3 STUDY DESIGN

3.1 General Description

This single center clinical trial is designed to determine the dose response of TSC on $tcpO_2$ following a single administration of TSC in subjects breathing O_2. Study assessments include $tcpO_2$ levels, SpO_2, and PK.

This is a randomized, double-blind, placebo-controlled study. A maximum of 30 healthy volunteers, aged 18-50 will be randomized to a single intravenous (IV) bolus dose of TSC (0.5, 1.0, 1.5, 2.0, or 2.5 mg/kg) or placebo (normal saline).

3.2 Treatments

The TSC will be administered as a one-time IV bolus injection to 5 unique subjects per dose cohort, to include 5 TSC dose levels and placebo. Placebo will consist of 7 mL of normal saline.

3.2.1 $TcpO_2$ Sensor Electrode Placement:

The $TcpO_2$ sensor electrodes will be applied to the left or right lower extremity. Four (4) sensors will be applied to the following locations:

- Sensor 1: Mid-dorsum of the foot
- Sensor 2: 10 cm distal to the lateral femoral epicondyle
- Sensor 3: 5 cm proximal to the anterior aspect of the lateral malleolus
- Sensor 4: 5 cm proximal from the center of the medial malleolus

3.2.2 O_2 Equilibration and Baseline Period:

After the $tcpO_2$ sensors have been applied and tested, subjects will be placed on O_2 via simple face mask at 6 L/minute, and will remain on O_2 for 70 minutes prior to study drug administration. At the end of the 70-minute equilibration/baseline period, subjects will continue on O_2 and receive a single IV bolus injection of TSC at a dose of 0.5, 1.0, 1.5, 2.0 or 2.5 mg/kg, or placebo.

After study drug administration, subjects will be evaluated for 60 minutes, with $tcpO_2$ values and SpO_2 recorded at 1, 2, and 5 minutes immediately post-dose and then every 5 minutes thereafter. In addition to the assessment of $tcpO_2$ as described, continuous $tcpO_2$ measurements will be recorded by the TCOM machine in graphical format.

3.3 Study Procedures

For complete details on the study assessments to be performed for each study period, refer to APPENDIX A.

3.4 Randomization and Unblinding Procedures

Volunteers will be randomized to one of 5 TSC doses or placebo in a 1:1:1:1:1:1 schema, with 5 subjects randomized to each of the 6 cohorts.

Subjects will be randomized to the following dose cohorts:

Dosing Cohort	# of Subjects in Cohort	TSC Dose (mg/kg)	Placebo (mL)
1	5	0.5	N/A
2	5	1.0	N/A
3	5	1.5	N/A
4	5	2.0	N/A
5	5	2.5	N/A
6	5	N/A	7 mL

N/A: Not Applicable

Each TSC dose will be calculated based on the subject's body weight in kg at the dosing level they are randomized to. Each individual dose of placebo will be normal saline 7 mL.

To maintain the double-blind, study drug administration will be performed by unblinded medical staff who will not be involved in other study procedures, including subject assessment. Subjects, investigators, and study coordinators will not see the injection or injection site or be aware of randomization.

Because TSC is highly colorized, this study will utilize unblinded and blinded personnel as follows:

- Unblinded study team: Pharmacist(s) and administering personnel
- Blinded study team: Investigator(s) and study coordinators

The unblinded study team will take specific steps to ensure that the subjects and blinded study personnel are not made aware of treatment assignment.

- Prior to release of the study drug from the pharmacy, the pharmacist will package the prepared syringe(s) in foil or acceptable masking material that masks the contents (i.e. cannot see through the material). The study drug should not be removed from the masking material until the time of administration to the subject.
- The medical staff assigned to administer the masked study drug will use a shroud to ensure that the subject is blinded to treatment. The shroud may be made of any material and be of any size as long as it sufficiently blocks the subject's view of the injection site and injection syringe at the time of administration. The shroud only needs to be in place at the time of study drug injection.
- A cloth or paper drape will be added underneath the injection site in the event that a droplet falls from the syringe as part of the injection process. The drape will be removed and folded up following the injection and before the shroud is removed so that it is not visible to the study team or subject.

- At the time of injection, the blinded study team must position themselves so that the injection site and syringe(s) are not visible during study drug injection. The subject will be asked to look away from the injection site at the time of study drug administration.

4 ANALYSIS POPULATION

Safety population will include healthy adult volunteers meeting all inclusion and exclusion criteria.

The following analysis populations will be used to summarize the results:

- Intent-to-Treat Population: The intent-to-treat (ITT) population includes all randomized subjects. Subjects in the ITT population will be analyzed as randomized.
- Per Protocol Population: The per protocol (PP) population includes subjects in the ITT population who do not have significant protocol deviations and complete the study.
  Protocol deviations will be assessed prior to database lock and release of the randomization codes.
  The PP population will be analyzed using the observed data for efficacy variables. Subjects in the PP population will be analyzed as treated, in the case there is an issue with the randomization assignment for the subject.
- Safety Population: The Safety population is synonymous with the ITT population.
- PK Population: The PK Population will include all subjects who have received at least one dose of the study drug and had at least 1 post-dose PK sample collection.
- PD Population: The PD Population will include all subjects in the ITT population who receive the study drug and have at least 1 pre-dose PD measurement prior to study drug dosing and at least 1 time-matched PD measurement after study drug dosing.

5 STUDY SUBJECTS

All recorded data will be presented in the data listings and summarized in table format. The disposition and protocol deviations will be presented for the safety population as described in Table 2.

Table 2: Data presentations for study subject information

Data	Variables	Presentation
Disposition and analysis population	Subject, completion status (i.e., completed or withdrawn), reason for withdrawal, analysis population determination	Listings: • Disposition, • Analysis population, • Randomization, Summary table including: • Number (N) of subjects enrolled, • Number (N) of subjects randomized, • N and %of subjects who completed the study, • N and % of subjects who discontinued from the study by primary reason for discontinuation and the overall number, independent of the reason for discontinuation. • N and % of subjects included in each of the study populations.
Protocol deviations	Protocol deviations	Listing: • General deviations

5.1 Disposition

Subject disposition will be summarized for all subjects enrolled in this study. The percentages will be calculated using the number of subjects randomized in each treatment group as the denominator. The number of subjects who complete the study, the number of subjects who prematurely withdraw from the study, and the reason for withdrawal, will be summarized by the randomized dose group and overall, independent of the randomized treatment assignment.

A listing of the disposition of all consented subjects will be provided. A listing of subjects included in each of the analysis populations will also be provided. A listing of the randomization assignment for each subject will also be presented.

5.2 Protocol Deviations

Deviations will be recorded in the clinic deviation tracking system (DTS) and presented as entered in a general protocol deviation listing and in a summary table as well.

6 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

All recoded data will be presented in the listings and summarize in table format for demographics and other baseline characteristics as described in Table 3.

Table 3: Data presentations for demographic and other baseline characteristics

Data	Variables	Presentation
Demographics, and Baseline characteristics	Sex, age, ethnicity, and race. Height, body weight at screening, and body mass index (BMI)	Listing and summary table
Lifestyle	Drinking and smoking habits	Listing
Medical history	All medical history findings	Listing Note: includes coded terms (system organ class [SOC] and preferred term [PT])
Prior medications	All medications taken prior to study drug administration (including prescription medications, nonprescription medications, dietary supplements, vitamins, or herbal medications)	Listing Note: includes coded terms (anatomic therapeutic chemical [ATC] level 1 and preferred name)
Contraception	Contraceptive method	Listing

7 PHARMACODYNAMIC ANALYSIS

The PD analysis will be performed on $tcpO_2$ pre dose and post dose values, and according to Altasciences SOPs.

7.1 $TcpO_2$ Data

$TcpO_2$ in subjects breathing oxygen (O_2) will be measured prior to study drug administration (baseline; Period 1) and following a single administration of TSC (Period 2) to determine the dose response of TSC on $tcpO_2$ levels.

Individual subject and summary of $tcpO_2$ levels will be listed.

7.2 Missing Values

The lack of $tcpO_2$ values due to failure to record the $tcpO_2$ measurement, a lost or compromised record or due to the subject's early termination from the study will be considered "missing" in the dataset, and no imputation will be done.

If the actual record time of a PD measurement is unknown, but a valid tcpO2 value has been recorded, the nominal record time will be used for the PD analysis and will be presented in listing excluded from descriptive statistics.

7.3 Measurements Below the Lower Limit of Quantitation

A non-detectable $tcpO_2$ value associated with baseline and postdose record times will be replaced with zero for PD analysis.

7.4 Nominal/Actual Record Times

For the time-matched change in $tcpO_2$ analysis, the non-compartmental analysis (NCA) on PD data will be based on the nominal record time. For the separate analysis of each study period, the NCA on PD data will be based on the actual record time.

The time-matched change in $tcpO_2$ levels vs time profiles, the mean $tcpO_2$ levels vs time profiles, the mean time-matched change in $tcpO_2$ levels vs time profiles and the tables presenting summary statistics of $tcpO_2$-time or time-matched change in $tcpO_2$ level-time data will be presented using nominal record times. The individual raw $tcpO_2$ levels vs time data will be presented using actual record times.

7.5 Change from Baseline Calculation

The baseline period is defined as the 60 minutes (following a 10-minute equilibration period) of O_2 delivery via a simple face mask prior to study drug administration.

The time-matched $tcpO_2$ change from baseline will be calculated by time point and subject using the following equation:

Time-matched change in $tcpO_2$ = ([$tcpO_2$, postdose, Time x] - [$tcpO_2$, baseline, Time x]).

Where Time x is the matching record time baseline and postdose.

7.6 Non-Compartmental Analysis

The following configuration for the NCA of $tcpO_2$ levels of each period and time-matched change in $tcpO_2$ levels (with Phoenix® WinNonlin® version 8, or higher) will be used:

- Data: Serial sampled data
- Model/Dose options Type: Plasma (200 -202) / Extravascular
- AUEC Calculation Method: Linear Trapezoidal/Linear Interpolation

The NCA will be performed using the median value from all sensors (calculated using the median value from each sensor).

The PD parameters for $tcpO_2$ levels of each period and time-matched change in $tcpO_2$ levels are defined in Table 4.

Table 4: Pharmacodynamic Parameters of $tcpO_2$ and Change in $tcpO_2$ Levels

PD parameter	Definition
E_{max}	Maximum observed effect
TE_{max}	Time of maximum observed effect; if it occurs at more than one time point, TE_{max} is defined as the first time point with this value
AUEC	Area under the effect-time curve from time 0 through 60 minutes postdose

7.7 Statistical Methodology

Specific algorithms are discussed for imputing missing or partially missing data, if deemed appropriate, under specific data topics. Imputed or derived data will be flagged in the individual subject data Listings. Imputed data will not be incorporated into any raw or primary datasets. The imputed data will be retained in the derived / analysis datasets.

The total duration for a subject *on-study* will be calculated as the difference between the date of the start of Period 1 to the last day of observation plus 1 day. All calculations for defining the duration on-study will follow the algorithm DURATION = [STUDY COMPLETION OR WITHDRAW DATE – PERIOD 1 START DATE + 1]. Extra measurements (such as unscheduled or repeat assessments) will not be included in the descriptive statistics, but will be included in subject listings.

Demographic and safety summary statistics will be presented for all subjects in the ITT / Safety Population. Data from subjects excluded from an analysis population will be included in the data listings, but not in the summaries.

For changes from baseline (pre-administration of study drug), only changes from baseline (pre-administration of study drug) to the defined post-administration time point will be shown in the listings and tabulations.

Listings will include all assessments (including repeated and unscheduled measurements) in chronological order with the scheduled measurements. Unscheduled and/or repeated measurements (unless used as the baseline measurement) will not be included in the tables. If any baseline (pre- administration) value is missing, the change from baseline will not be calculated.

This section details general conventions to be used for the statistical analyses. The following conventions will be applied to all data presentations and analyses.

- Summary statistics will consist of the count, percentage, and exact 95% and 97.5% binomial confidence intervals, depending on the variable, at each level for categorical variables, and the sample size (n) mean, median, standard deviation (SD), minimum, and maximum values for continuous variables.

- All mean and median values will be formatted to one more decimal place than the measured value. Standard deviation values will be formatted to two more decimal places than the measured value. Minimum and maximum values will be presented with the same number of decimal places as the measured value.

- The number and percentage of responses will be presented in the form XX (XX.X%).

- All probability values will be rounded to four (4) decimal places. All p-values that round to 0.0000 will be presented as '<0.0001' and p-values that round to 1.0000 will be presented as '>0.9999'. P-values <0.05 will be considered to be statistically significant.

- All summary tables will include the analysis population sample size (i.e., number of subjects with values for the analysis).

- Study Day 1 is defined as the day the subject receives the study drug. All *study days* are determined relative to the day of the administration of study drug.

- Baseline values will be defined as those values recorded closest to, but prior to, the administration of study drug.

- Change from baseline will be calculated as follows:
    - Change = Post-baseline value – baseline value.

- Date variables will be formatted as DDMMMYYYY for presentation.

- Tables, figures, and Listings will be presented in landscape orientation.

- SAS® Version 9.4 will be the statistical software package used for all data analyses.

- All data from this study will be presented in Listings. All Listings will be sorted by clinical site, subject number, and visit date, as applicable.

Table and Listing numbering will follow ICH guidelines for post-text table and Listing numbering.

The header of each table and Listing will include the sponsor's name and the study number. The information and explanatory notes to be provided in the "footer" or bottom of each table and Listing will include the following information:

1. Date and time of output generation.
2. SAS® program name, including the path that generates the output.
3. Any other output specific details that require further elaboration.

In general, tables will be formatted with a column displaying findings for all subjects combined. Row entries in tables are made only if data exist for at least one (1) subject (*i.e.*, a row with all zeros will not appear). The only exception to this rule applies to tables that list the termination status of subjects (*e.g.*, reasons for not completing the study). In this case, zeros will appear for study termination reasons that no subject satisfied. The summary tables clearly indicate the number of subjects to which the data apply and unknown or not performed are distinguished from missing data. Tables, Listings, and figures will provide the units of measurement, unless not applicable.

All summaries of AEs will be based on events that occurred during the study. AEs will be mapped to preferred terms and body systems using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary. The number and percentage of subjects experiencing AEs will be summarized by system organ class and preferred term. Summaries by maximum severity and relationship to the study device and procedure will also be provided. SAEs and AEs leading to discontinuation from the study will be presented by system organ class and preferred term.

All tables, figures and listings, when appropriate, will be stratified by treatment.

7.7.1 Summary Statistics

Summary statistics of the individual $tcpO_2$ levels, time-matched $tcpO_2$ change from baseline levels and derived parameters will be calculated with the built-in Phoenix® WinNonlin® platform for the PD population.

Summary statistics will be calculated for $tcpO_2$ levels and time-matched $tcpO_2$ change from baseline levels at each individual time point and for all PD parameters. Individual $tcpO_2$ levels, time-matched $tcpO_2$ change from baseline levels, and derived PD parameters obtained from the NCA will be summarized using the following descriptive statistics: number of observations (N), minimum, arithmetic mean, median, maximum, standard deviation (SD), and CV%.

7.8 Analysis of the Primary Endpoint

Analysis of Change Over Time in $tcpO_2$:

The primary analysis will be based on the change over time in the $tcpO_2$ measurements following a single administration of TSC in subjects breathing O_2. The time-matched changes in 5 minute increments for Period 1 (60 minute run-in on O_2) to Period 2 (60 minute post-drug administration on O_2) in 5 minute increments will calculated for each subject. The first 5 minute interval in Period 1, following the 10 minute equilibration period, will be matched to the first 5 minute interval in Period 2. This same approach will be followed for the remaining eleven 5 minute intervals.

The median value for each sensor will be calculated using the 10 second readings within a 5 minute interval, calculated separately for Period 1 and Period 2. The median will be calculated using the median value from each sensor.

The intra-subject changes (Period 2 minute Period 1 in 5 minute intervals) will serve as the dependent variable in the repeated measures analysis. The 60 minute run-in on O_2 is intended to account for the intra-subject variability over time and the time-match differences represent the least biased estimate of the effect of the study drug. With 5 subjects per randomized treatment arm, the change over time should provide sufficient clinical information to determine if there is a drug effect, and if the effect in an active drug treatment arm is different than placebo.

To compare the time-match changes in $tcpO_2$, a 2-factor (treatment and time) repeated measures (time) analysis of variance (ANOVA) model will be used. Contrast statements within the model will facilitate comparisons between individual treatment arms over individual time points, providing guidance relative to the initial time and duration of separation.

Analysis of AUEC Comparison:
The trapezoidal rule will be used for the construct of the area under the curve (AUEC) analyses. The AUEC based on the time-matched differences, a 1-factor (treatment) analysis of variance model will be used, followed by a Dunnett's test to compare the least square mean AUEC values from the 5 active drug groups relative to the placebo group.

A separate AUC analysis will be performed, calculating the AUC for Period 1 and Period 2 for each subject. The intra-subject difference (AUC from Period 2 minus the AUC from Period 1) will be analyzed using a repeated measures (Period) analysis of covariance (Period 1 AUC) followed by a Dunnett's test to compare the least square mean AUC values from the 5 active drug groups relative to the placebo group.

If the data within a treatment arm is skewed, suggesting that the least square mean value is not the best measure of central tendency, the data may be ranked and the analyses will be conducted using the ranked scores.
Information regarding the examination of the distribution of the data
To compare the time to the maximum increase in $tcpO_2$, the event time distribution functions will be compared using a log rank test and presented using Kaplan-Meier curves.

All probability values will be 2-sided and accepted at face value for this feasibility study; there will not be an adjustment for multiplicity. For interpretation, probability values <0.05 will be considered significant; probability values <0.1 but ≥ 0.05 will be considered highly suggestive of a significant difference.

8 SAFETY ANALYSIS

All data will be listed and summary tables for safety assessments will be presented for the safety population as detailed in Table 5.

Table 5: Data presentations for safety assessments

Data	Variables	Presentation
Adverse events	Adverse event (AE) description, date and time, severity, relationship to study drug, action taken, and outcome	Listings, summary tables by randomized treatment , time, and overall, including number and percentage of subjects experiencing: • Reported AEs, • Treatment-emergent adverse events (TEAEs), • Drug-related TEAEs (i.e. those with a relationship classified asRelated), • TEAEs by relationship to study treatment (i.e. related or not related), • TEAEs by severity, • Serious TEAEs, • Study drug-related serious TEAEs, • TEAEs leading to withdrawal, and • TEAEs with an outcome of death. Note: includes coded terms (system organ class [SOC] and preferred term [PT])
Concomitant medications	All medications taken during the study (as defined in the protocol and including prior medications that are continued), dose, units, frequency, formulation, route, indication/ reason taken, and start and end date and time	Listing Note: includes coded terms (anatomic therapeutic chemical [ATC] level 1 and preferred name)
Extent of exposure	Category of treatment, Study drug administration dose, date, time, formulation, frequency, route and compliance questions (if applicable)	Listing
Clinical laboratory evaluations	Laboratory results (refer to section 8.2 for parameters)	Listings: • All laboratory values by category, • All out-of-range laboratory values, and • All clinically significant laboratory values

Data	Variables	Presentation
Vital signs	Blood pressure, pulse, respiratory rate and body temperature	Listings: • All vital signs values, and • Clinically significant vital signs values Summary tables by randomized treatment and overall.
Physical examination	Physical examination findings	Listings: • All physical examination findings, and • Clinically significant physical examination findings
Electrocardiograms (ECGs)	ECG interpretations and findings	Listings: • All ECGs, and • Clinically significant ECGs

Note: Sub-bullets denote individual listings or tables to be generated.

8.1 Adverse Events

An AE is defined as any untoward medical occurrence in a clinical investigation subject administered an study drug and which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a study drug, whether or not related to the study drug.

Treatment emergent adverse events (TEAEs) are AEs not present prior to the exposure to study treatment or AEs already present that worsen in intensity or frequency following exposure to study treatment. All AEs reported following exposure to study treatment are considered TEAEs.

All TEAEs will be assigned to a treatment using the following rules:

- A TEAEs will be assigned to the last drug taken by the subject where the date and time of the last drug dosing is on or before of the start date and time of the event. Such assignment will be performed irrespective of any washout period between the start and stop dates of the TEAE.
- Any TEAE started during the follow-up period will be assigned to the last treatment that the subject has taken.

In case that the time of onset or time of resolution is unknown, worst case scenario will be considered. For the onset, 00h01 will be the time considered. For the resolution time, if the time is unknown, 23h59 will be the time considered.

Frequency tables summarizing all TEAEs and all study drug-related TEAEs (including SOC and PT) will be presented by treatment and overall as described in **Error! Reference source not found.**.

Adverse events will be coded using MedDRA and summarized by system organ class and preferred term and presented by randomized treatment assignment, Period 1 (60 minute run-in on O_2) or Period 2 (60 minute post-drug administration on O_2), and overall, independent of Period.

8.2 Clinical Laboratory Evaluations

Laboratory data will be listed for abnormal results and clinically significant (CS) abnormal results.

Laboratory data will be presented using units as reported by the clinical laboratory.

The following clinical laboratory tests listed will be performed as scheduled in 11APPENDIX A.

CBC	White blood cells (WBC), red blood cells (RBC), Hemoglobin, Hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), Platelet Count, mean platelet volume (MPV) and Differential (Absolute and Percent - Neutrophils, Lymphocytes, Monocytes, Eosinophils, and Basophils)
BMP	BUN/Creatinine Ratio (calculated), Calcium, Carbon Dioxide, Chloride, Creatinine with GFR Estimated, Glucose, Potassium, Sodium, Urea Nitrogen (BUN)
Serology	HIV-1/HIV-2 Antibodies (HIV), Hepatitis B surface Antigen (HBsAg), Hepatitis C Antibody (HCVAb)
Pregnancy	Serum pregnancy test at screening, urine pregnancy test at clinic arrival
Urine Drug Screen	Amphetamines, barbiturates, benzodiazapenes, cannabinoids, cocaine, opiates, phencyclidine
Alcohol Screen	Breathalyzer

8.3 Vital Signs

Vital signs will include systolic and diastolic blood pressure, heart rate, and temperature.

Values will be listed and summarized by time points for each treatment group, and overall. Changes from baseline will also be presented.

Subject listing of vital signs values, clinically significant on-study vital signs and abnormal on-study vital signs values will also be provided.

8.4 Physical Examination Findings

Physical examination includes a review of the following: head, eyes, ears, nose, throat (HEENT), neck, chest, back, abdomen, extremities and neurological function. All physical examination records will be presented in a listing, by subject, and visit. Similarly, clinically significant on-study physical examinations will be presented in a second listing.

8.5 12-Lead ECG Findings

Subject listings will be provided for all ECG parameters, and clinically significant (CS) abnormal results.

9 DATA HANDLING AND PRESENTATION

All safety and statistical outputs will be generated using SAS software, version 9.4.

All programs used to generate statistical analyses will be validated according to Altasciences's standard operating procedures (SOPs).

The analyses described in this plan are considered a priori, in that they have been defined prior to database lock and prior to breaking the blind. Any analyses performed subsequent to database lock and breaking the blind that are not described within the present plan will be considered post hoc and exploratory. Post hoc analyses will be labeled as such in the corresponding statistical output and identified in the clinical study report (CSR).

9.1 Safety Analysis Presentation

Adverse events and medical history will be classified using the Medical Dictionary for Regulatory Activities (MedDRA) terminology version 23.1 as defined in the study data management plan (DMP) dated 2021/03/01.

Prior and concomitant medications will be coded with the World Health Organization Drug Dictionary- Enhanced (WHO-DDE) as defined in the study DMP.

In general, all safety summary tables will be presented for the safety population. Summaries for AEs will be presented by treatment and Overall. Summaries for other safety endpoints will be presented by treatment if the endpoints are measured at the end of each period.

In general, the data listings will include all randomized subjects up to the point of study completion or discontinuation; exceptions will be listings pertaining to a subset of subjects only (e.g., subjects with protocol deviations) or a subset of records/events (e.g., abnormal laboratory values).

Categorical variables will be summarized using the PROC FREQ procedure. Continuous variables will be summarized using the PROC UNIVARIATE procedure. For natural log (ln)-transformed endpoints, geometric mean, geometric standard deviation (SD), and coefficient of variation (CV%) will also be presented.

The following general comments also apply to all statistical analyses and data presentations:

- Duration variables will be calculated using the general formula: (end date - start date) +1.
- If the reported value of a clinical laboratory parameter cannot be used in a statistical summary table (e.g., a character string is reported for a parameter of the numerical type), a coded value must be appropriately determined and used in the statistical analyses. In general, a value or lower and upper limit of normal range such as '<10' or '≤5' will be treated as '10' or '5' respectively, and a value such as '>100' will be treated as '100'. However, the actual values as reported in the database will be presented in data listings.
- When assessments are repeated for a given time point or performed at unscheduled times, only the result which is the closest to the dosing time will be included in summary tables.

In general, summary statistics for raw variables (i.e., variables measured at the study site or central laboratory) will be displayed as follows:

- Minima and maxima will be displayed to the same number of decimal places as the raw data.
- Means, medians, and quartiles will be displayed to 1additional decimal place.
- Standard deviations will be displayed to 2 additional decimal places.
- Percentages will be displayed to 1 decimal place. Percentages between 0 and 0.1 (exclusive) will be displayed as '<0.1'.
- P-values will be displayed to 3 decimal places. P-values that are less than 0.001 will be displayed as '<0.001'.

The numbers of decimal places for summary statistics of derived variables (i.e., variables that are not measured by the study site but are calculated for analysis based on other measured variables) will be determined on a case by case basis. In general:

- Minima and maxima will be displayed to the commonly used unit of precision for the parameter.
- Means, medians, quartiles, and confidence limits will be displayed to 1 additional decimal place.
- Standard deviations will be displayed to 2 additional decimal places.

9.2 Pharmacokinetic Analysis

Individual raw PK concentrations will be displayed with the same precision as received from the bioanalytical laboratory.

Summary statistics for concentration will be displayed with the same precision as the individual values, with the exception of number of observations (N) and CV% which will be presented with 0 and 1 decimal place, respectively.

9.3 Pharmacodynamic Analysis

Precision for individual PD parameters will be displayed as follows:

- E_{max}, AUEC with the same precision as the raw PD data;
- PD parameters associated with time (i.e. TE_{max}) with 2 decimal places;

Summary statistics for $tcpO_2$ levels, time-matched change in $tcpO_2$ levels and PD parameters will be displayed with the same precision as the individual values unless specified otherwise, with the exception of N and CV% which will be presented with 0 and 1 decimal place, respectively.

9.4 Analysis Timepoints

Unless otherwise specified, the baseline value will be defined as the last non-missing evaluation prior to the first dose of study medication.

9.5 Methods for Handling Missing Data

All analyses will be based on the observed data; no data will be imputed for the statistical analyses.

9.6 Interim Analysis

No interim assessments or analyses are planned for this study.

10 CHANGES TO PROTOCOL-SPECIFIED ANALYSES

Addition of the PD and PK population in the analysis population. Clarification of the PD analysis to be performed.

11 GENERAL INFORMATION RELATED TO DATA PRESENTATIONS

The formats and layouts of TFLs are provided in a separate document and are common displays. Their numbering and general content follow the International Conference on Harmonisation (ICH) E3 guidelines. Actual formats and layouts may be altered slightly from those presented as necessary to accommodate actual data or statistics. Minor format changes will not require updates to the SAP, rather they may be documented in a Note to SAP.

APPENDIX A STUDY SCHEDULE

	Screening	Treatment (Day 0)					Follow Up
	Day -21 to Day 0	**Clinic Arrival**	**Equilibration/ Baseline: 70 minutes on O_2**	**Treatment/ Evaluation: 60 minutes on O_2**	**Post-Treatment: 30 minutes on Room Air**	**Clinic Observation**	**48 Hours (+ 2 Days)**
Informed Consent	X						
Inclusion/Exclusion criteria	X	X					
Demographics	X						
Medical history	X	X					
Concomitant medications	X	X	X	X	X	X	X
BMI (kg/m^2)	X						
Body weight (kg)		X					
Physical Exam	X	X					
Randomization		X					
Vital signs (HR, BP, RR, Temp)	X		X	X	X	X	
SpO_2 [b]	X		X	X			
12-lead ECG	X						
Laboratory (CBC, BMP, HIV, Hep B, Hep C)	X						
Pregnancy test[a]	X	X					
Urine drug screen	X	X					
Alcohol breathalyzer screen	X	X					
Subject resting quietly supine with one pillow, or semi-recumbent			X	X			
$TcpO_2$ sensors in place		X	X	X			
PK measurements			X	X	X		

Oxygen: simple face mask at 6 L/min			X	X			
$TcpO_2$[b]			X	X			
Administer Study Drug				X			
$TcpO_2$ sensors removed					X		
Overnight stay at clinic						X	
Adverse Events			X	X	X	X	X

[a] For all females, with serum pregnancy test at screening and urine pregnancy test at clinic arrival

[b] SpO2 and TcpO2 will be assessed every 5 minutes during the baseline period. After study drug administration they will be assessed at 1, 2, and 5 minutes, and then every 5 minutes thereafter.

ALTASCIENCES

STATISTICAL ANALYSIS PLAN APPROVAL

We have carefully read this statistical analysis plan and agree it contains the necessary information required to handle the statistical analysis of study data.

Sanaa Boudhar Senior Biostatistician Altasciences Company Inc	Date
Dominique St-Fleur Senior Pharmacokinetic Scientist Altasciences Company, Inc.	Date

On behalf of the Sponsor:

Sponsor Representative	Date
Bruce C. Stouch, Ph.D. ~~Director, Biostatistics~~ BIOSTATISTICIAN	8 APR 2021 Date