MEDPACE

STATISTICAL ANALYSIS PLAN

Protocol Title:	**A Double-Blind, Randomized, Placebo-Controlled Study of Levosimendan in Pulmonary Hypertension Patients with Heart Failure and Preserved Left Ventricular Ejection Fraction (PH-HFpEF)**
Protocol Number:	TNX-LVO-04
Protocol Version/Date:	Version 4.0, 15 May 2019
Investigational Product:	Levosimendan (Simdax) Injection
Sponsor:	Tenax Therapeutics, Inc. One Copley Parkway Morrisville, NC 27560
SAP Version/Date:	Version 2.0, 18 May 2020

SIGNATURE PAGE

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We, the undersigned, have reviewed and approved this Statistical Analysis Plan:

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VERSION HISTORY

Version	Version Date	Description
1.0	09 March 2020	Original signed version
2.0	18 May 2020	Update the Per-Protocol Set definition

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LIST OF ABBREVIATIONS

Abbreviation	Definition
6MWT	6-Minute Walk Test
AE	Adverse Event
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CSR	Clinical Study Report
COVID-19	Coronavirus Disease 2019
eCRF	Electronic Case Report Form
ECG	Electrocardiogram
HFpEF	Heart Failure with Preserved Ejection Fraction
IRT	Interactive Response Technology
LLN	Lower Limit of Normal
LV	Left Ventricular
MedDRA	Medical Dictionary for Regulatory Activities
NYHA	New York Heart Association
PAP	Pulmonary Artery Pressure
PCWP	Pulmonary Capillary Wedge Pressure
PH	Pulmonary Hypertension
PK	Pharmacokinetic
PVR	Pulmonary Vascular Resistance
RAP	Right Atrial Pressure
RV	Right Ventricle
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SD	Standard Deviation
TAPSE	Tricuspid Annular Plane Systolic Excursion
TEAE	Treatment-Emergent Adverse Event
ULN	Upper Limit of Normal
WHODrug	World Health Organization Drug

1 INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to provide a description of the statistical methods to be implemented for the analysis of data from the study with protocol number TNX-LVO-04. The SAP will be finalized prior to database lock. Any deviations from the SAP after database lock will be documented in the final Clinical Study Report (CSR).

2 STUDY OVERVIEW

2.1 Study Objectives

2.1.1 Primary Objectives

This study will evaluate the efficacy and safety of intermittent (weekly) levosimendan infusions in hemodynamic improvement with exercise in Pulmonary Hypertension with Heart Failure and Preserved Ejection Fraction (PH-HFpEF) subjects.

2.1.2 Secondary Objectives

The study will evaluate the effectiveness of intermittent (weekly) levosimendan infusions in improving the following:

- Change in Cardiac Index at rest and with exercise at Week 6
- Change in pulmonary vascular resistance (PVR) effect at rest and with exercise at Week 6
- Change in pulmonary capillary wedge pressure (PCWP) when supine and legs elevated at Week 6
- Patient global assessment (based on a six-point Likert scale) at Week 6
- Exercise duration 6-min walk test at Week 6
- Physician's Assessment of Functional Class
- Clinical Events: Death and hospitalizations
    - All cause hospitalizations
    - Hospitalizations attributed to any abnormality in cardiac and/or pulmonary status.

2.1.3 Exploratory Objectives

Baseline echocardiographic measurements of right ventricle (RV) and left ventricle (LV) function to predict favorable response to levosimendan therapy.

2.2 Study Design

2.2.1 Overview

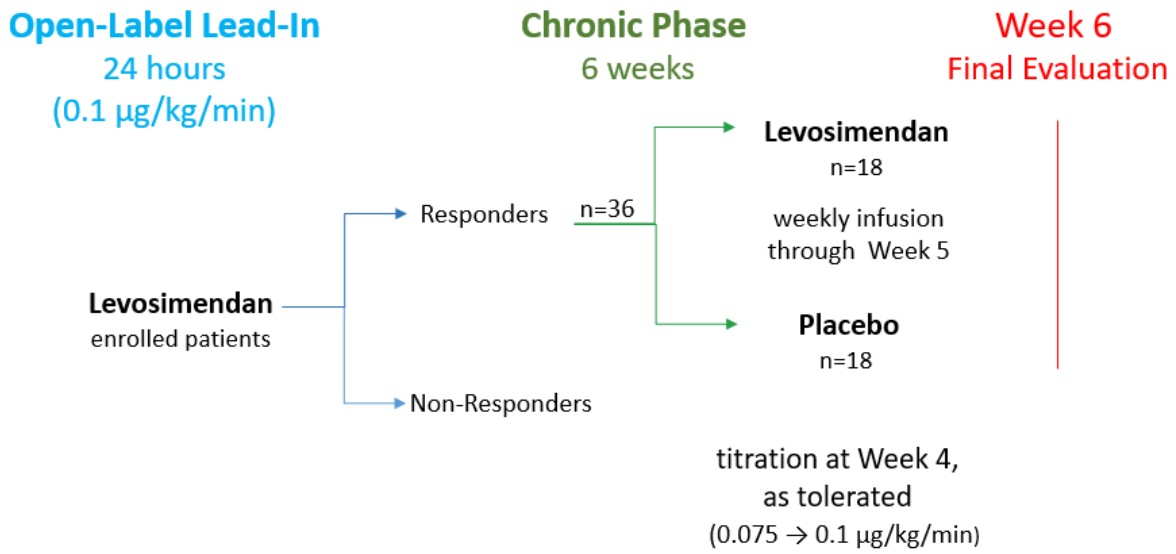
This study will be conducted at approximately 20-25 sites in the United States. The Phase 2 study of levosimendan in PH-HFpEF subjects has been designed in two phases (Figure 1). The study will recruit male and female subjects meeting identified enrollment criteria for PH-HFpEF into the lead-in open label phase to identify "responders" to levosimendan intravenous infusion of at 0.10 µg/kg/min for 24 hours ±30 min. Subjects that have clinically relevant responses to levosimendan will be randomized to levosimendan or placebo (1:1) and receive intermittent (weekly) study drug infusions (Week 2-5). The first 36 subjects who demonstrate a ≥4mmHg reduction in PCWP from baseline measured at bicycle exercise (25 watts) with no more than a 10% decrease from baseline in cardiac index will be classified as a "responder" to the

lead-in levosimendan dose and be randomized to the double-blind phase of the study. Patients who do not tolerate the initial levosimendan infusion due to excessive hypotension or tachycardia will be dropped from eligibility. In the double-blinded phase, patients will be randomized to levosimendan or placebo and receive a weekly dose of study drug intravenously at 0.075 µg/kg/min for 24 hours ±30 min and if tolerated titrated to 0.10 µg/kg/min for 24 hours ±30 min at Week 4. The infusion rate of study drug may be decreased or interrupted as clinically warranted if the subject has hypotension, tachycardia, or signs or symptoms consistent with hypovolemia (e.g., low systolic blood pressure [SBP], decreasing urine output with rising blood urea nitrogen [BUN] and serum creatinine) Record the time of discontinuation or down titration. Patients should be monitored closely until clinically stable. See Protocol Attachment 1 for infusion rates and instructions on dose adjustments. Patients will be evaluated Week 6 to determine the efficacy and safety of levosimendan. Randomized subjects that have not received study drug weekly through Week 5 and returned for evaluation on Week 6 will be replaced to ensure 18 subjects have completed in each study group.

If a subject terminates the study prematurely, procedures listed in the Week 6 visit should be performed as soon as possible. The RHC, echocardiogram, and 6-minute walk test (6MWT) do not need to be performed for subjects who terminate the study early. Subjects will be followed for safety for 2 weeks after early termination. A phone contact will be made with the subject at 24 hours (+ 24 hours), 7 days (+ 2 days), and 14 days (+ 2 days) post infusion by investigational site personnel to assess any new or ongoing adverse events. If a subject discontinues greater than 24 hours after their prior infusion, the 24-hour phone contact does not need to be performed and the next phone contact beginning at 7 days should be completed. The vital status of all subjects will be collected at the end of the trial, regardless of reason for discontinuation.

Refer to Protocol for the time and events schedule of the study procedures.

Figure 1: Study Design Flow Chart



2.2.2 Blinding

The blind is maintained by the use of matching placebo.

All subjects are to remain blinded until all subjects have completed the study (defined as Week 6) and the database is locked. The protocol specifies that the blind could be broken only if specific emergency treatment would be dictated by knowing the treatment status of the subject. In such cases, the investigator must contact the sponsor or designee. Please consult the Pharmacy Manual for unblinding procedures should the sponsor or designee be unavailable. The sponsor must be informed as soon as possible. The date, time, and reason for the unblinding must be documented in the appropriate section of the electronic Case Report Form (eCRF) and in the source document.

2.2.3 Sample Size Calculation and Power Considerations

The sample size was estimated for the primary comparison of levosimendan and placebo using SAS (v9.4) for windows procedure PROC POWER.

A total of 36 randomized subjects (at 1:1 levosimendan vs. placebo) will provide 80% power to detect the difference at two-sided 0.05 level, assuming a standard deviation (SD) of 5 mmHg in PCWP and a treatment difference of ≥4.9 mmHg.

2.3 Study Endpoints

2.3.1 Primary Efficacy Endpoint

- Change from baseline PCWP during bicycle exercise (25 watts) at Week 6

2.3.2 Secondary Efficacy Endpoints

- Change in Cardiac Index at rest and with exercise at Week 6
- Change in PVR effect at rest and with exercise at Week 6
- Change in PCWP when supine and legs elevated at Week 6
- Change in mean pulmonary artery pressure (mPAP) effect at rest and with exercise at Week 6
- Change in right atrial pressure (RAP) effect at rest and with exercise at Week 6
- Patient global assessment (based on a six-point Likert scale) at Week 6
- 6MWT at Week 6 (distance)
- Physician's Assessment of Functional Class
- Clinical Events: Death and hospitalizations

2.3.3 Exploratory Efficacy Endpoint

- Change in echo measurements of RV size and tricuspid annular plane systolic excursion (TAPSE)

2.3.4 Safety Endpoints

- Adverse Event
- Events of Special Interest
- Clinical Laboratory Evaluations
- Vital Signs
- Electrocardiogram

- Cardiac monitoring data (baseline, Week 5)

3 STATISTICAL METHODOLOGY

3.1 General Considerations

3.1.1 Study Day

Study day will be calculated from the date of first dose of study drug (lead-in infusion). The day of the first dose of study drug will be Day 1, and the day immediately before Day 1 will be Day -1. There will be no Day 0.

3.1.2 Definition of Baseline

Baseline is defined as the value obtained prior to lead-in study drug infusion on Visit 1. Note: The protocol-specified visit 'Day 0' will be referred to as 'Visit 1' for analysis purposes.

3.1.3 Analysis Visit Windows

Post-baseline assessments will be assigned a visit number based on the study day. Analysis visit windows will be defined as below:

- Screening: ≤28 Days Prior to Visit 1,
- Lead-In Visit: Visit 1/Week 1/Day 1,
- Visit 2/Week 2/Day 8: Day 2 to Day 11,
- Visit 3/Week 3/Day 15: Day 12 to Day 18,
- Interim Office Visit: Between Visit 3/Week 3 and Visit 4/Week 4,
- Visit 4/Week 4/Day 22: Day 19 to Day 25,
- Visit 5/Week 5/Day 29: Day 26 to Day 32, and
- Visit 6/Week 6/Day 36: Day 33 or later.

3.1.4 Summary Statistics

Categorical data will generally be summarized with counts and percentages of subjects. The denominator used for the percentage calculation will be clearly defined. Continuous data will generally be summarized with descriptive statistics including n (number of non-missing values), mean, median, standard deviation, minimum, and maximum.

3.1.5 Handling of Dropouts and Missing Data

In cases of missing or incomplete data (e.g. adverse event [AE] and concomitant medications), the missing component(s) will be assumed as the most conservative value possible. For example, AEs with missing start dates, but with stop dates either overlapping into the treatment period or missing, will be counted as treatment-emergent, taking the worst-case approach. When partial dates are present in the data, both a partial start date and/or a partial stop date will be evaluated to determine whether it can be conclusively established that the AE started prior to the start of study drug or ended prior to the start of study drug. If the above cannot be conclusively established based on the partial and/or present dates, then the AE will be considered as treatment-emergent. Actual data values as they appear in the original eCRF will be presented in the data listings. Missing values for other variables will not be imputed and only observed values will be used in data analyses and presentation.

3.2 Analysis Populations

3.2.1 Screened Set

The Screened Set is defined as subjects who signed informed consent.

3.2.2 Enrolled Set

The Enrolled Set is defined as subjects who signed informed consent and met open-label lead-in inclusion-enrollment criteria.

3.2.3 Randomized Set

The Randomized Set is defined as enrolled subjects who met all the inclusion-randomization criteria as responders to levosimendan for whom a randomization number was assigned.

3.2.4 Safety Analysis Set

The Safety Analysis Set is defined as enrolled/randomized subjects who received open-label levosimendan and/or any blinded study drug.

3.2.5 Full Analysis Set

The Full Analysis Set is defined as randomized subjects who received any blinded study drug and completed the study with Week 6 office visit.

3.2.6 Per-Protocol Set

The Per-Protocol Set is defined as subjects in the Full Analysis Set who completed the final scheduled primary assessment for the study and did not have pre-defined major protocol deviation.

Major protocol deviations may include but are not limited to:

- Did not meet recruitment inclusion criteria
- Did not complete the Week 6 office visit (or final office visit as rescheduled due to Coronavirus Disease 2019 [COVID-19])
- Missed more than one study drug infusion
- Did not have baseline and Week 6 (or final office visit as rescheduled due to COVID-19) data for primary endpoint

3.2.7 Pharmacokinetic Set

The Pharmacokinetic Set is defined as subjects in the Full Analysis Set for whom the primary pharmacokinetic data is considered sufficient and interpretable.

3.3 Subject Data and Study Conduct

3.3.1 Subject Disposition

Subjects in each analysis population, as well as subjects who complete the study, and subjects who prematurely discontinue from the study will be summarized by treatment assignments in the double-blind phase using descriptive statistics. In addition, for subjects who prematurely discontinue from the study, the reasons for discontinuation will be summarized by treatment group.

Subjects who discontinue the study prior to the Week 6 office visit will be replaced to ensure 18 randomized subjects in both arms (levosimendan, placebo) have completed the study for the primary analysis. If a subject has been identified for replacement, the Interactive Response Technology (IRT) will select the lowest available randomization number (with the same treatment group as the subject being selected) in the

"Replacement Blocks" section of the randomization schedule for the treatment assignment of the replacement subject. Thus, the treatment assignment of both the subject being replaced and the replacement subject will remain blinded.

3.3.2 Protocol Deviations

CSR reportable protocol deviations as defined in the Medpace Protocol Deviation Plan will be summarized for the Safety Analysis Set by treatment assignments in the double-blind phase and in total. The number and percentage of subjects with deviations will be summarized by deviation and overall.

A data listing of all CSR reportable protocol deviations will be provided by subject.

3.3.3 Demographic and Baseline Characteristics

The following demographic and baseline characteristics will be summarized:

- Age (years) and age categories (<65 years, ≥65 years)
- Sex
- Race
- Ethnicity
- Height (cm)
- Weight (kg)
- Body mass index (BMI) (kg/m^2)
- Baseline hemodynamic parameters (PCWP, Cardiac Index, PVR, PAP (mean), RAP)
- Baseline echo measurements
- Baseline 6-Minute Walk Test (exercise distance) (m)

Demographic and baseline characteristics will be summarized with descriptive statistics or counts and percentages of subjects as appropriate by treatment assignments in the double-blind phase and in total for the Safety Analysis Set and Full Analysis Set.

3.3.4 Medical History

Medical history will be coded to system organ class and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA) version 21.0. Counts and percentages of subjects with medical history by system organ class and preferred term will be summarized by treatment assignments in the double-blind phase and in total based on Safety Analysis Set.

3.3.5 Concomitant Medications

Concomitant medications will be coded to anatomical therapeutic chemical (ATC) class and preferred term using the World Health Organization Drug (WHODrug) Dictionary Mar 2018 Global B3 version. For summary purposes, medications will be considered prior medications if they stopped prior to the first dose of study drug (lead-in infusion) and concomitant medications if they were taken at any time after the first dose of study drug (lead-in infusion) (i.e. started prior to the first dose of study drug and were ongoing or started after the first dose of study drug).

Counts and percentages of subjects taking prior and concomitant medications by ATC class and preferred term will be summarized by treatment assignments in the double-blind phase and in total based on the Safety Analysis Set.

3.3.6 Study Duration and Drug Exposure

Study duration will be calculated as the date of last visit – date of first dose (lead-in phase) + 1. Study duration will be summarized with descriptive statistics by treatment assignments in the double-blind phaseand in total. Additionally, study duration will be categorized according to the following categories:

- 1 to 15 days,
- 16 to 29 days, and
- >29 days.

A summary of subjects receiving the study drug will be provided by treatment assignments in the double-blind phase and in total for each visit on the Safety Analysis Set and Full Analysis Set.

The number of infusions for each subject will be summarized with counts and percentages by treatment assignments in the double-blind phase and in total on the Safety Analysis Set and Full Analysis Set.

3.4 Efficacy Assessment

All efficacy analyses will be based on the Full Analysis Set and all statistical tests will be 2-sided hypothesis tests performed at the 5% level of significance. Also, all confidence intervals will be 2-sided 95% confidence intervals, unless stated otherwise. All the efficacy analyses will be performed on the assigned treatments in the double-blind phase.

3.4.1 Primary Efficacy Endpoints

Primary Analysis

The primary efficacy variable is defined as the change from baseline PCWP with bicycle exercise (25 watts) at 6 weeks. The baseline PCWP with bicycle exercise (25 watts) is defined as the value obtained prior to lead-in study drug infusion at Visit 1.

The primary efficacy analysis will be conducted over the Full Analysis Set. The PCWP will be analyzed using an analysis of variance (ANOVA) model with change from baseline to Week 6 in PCWP as dependent variable, treatment group as a factor.

An example of SAS code is provided below:

```
**********************************************************************
** PCWP = Change in PCWP from Baseline                               **
** TREATMENT = Treatment group where 0 if Placebo, 1 if Levosimendan **
**********************************************************************
PROC GLM;
  CLASS TREATMENT;
  MODEL PCWP = TREATMENT;
  LSMEANS TREATMENT / E PDIFF CL STDERR ALPHA=0.05;
  ESTIMATE 'LEVOSIMENDAN VS PLACEBO' TREATMENT 1 -1 / E;
RUN;
```

Sensitivity Analyses

Sensitivity Analyses 1: A sensitivity analysis will include an adjustment for baseline. The PCWP will be analyzed using an analysis of covariance (ANCOVA) model with change from baseline to Week 6 in PCWP as dependent variable, treatment group as a factor and baseline as a covariate.

Sensitivity Analyses 2: A sensitivity analysis by acetylation status. The PCWP with bicycle exercise (25 watts) at 6 weeks will be analyzed using an ANOVA with change from baseline to Week 6 in PCWP as dependent variable, treatment group and the acetylation status (fast, intermediate, and slow) as factors.

Supportive Analyses

An additional primary endpoint analysis will be repeated on the Per-Protocol Set.

3.4.2 Secondary Efficacy Endpoints

The secondary efficacy analyses will be performed on the Full Analysis Set.

Analyses of hemodynamic endpoints will be performed using the same ANOVA model as the primary endpoint. Series Plots will be provided for PCWP by position and visit.

Patient global assessment (based on a six-point Likert scale) at Week 6 will be performed by a Mann-Whitney-Wilcoxon test for each of the six symptom's grade. Total score will be calculated as the sum of all 6 scores for the subjects with all 6 scores available.

6MWT exercise distance at Week 6 will be tested using the same ANOVA model as the primary endpoint.

Physician's assessment of New York Heart Association (NYHA) functional class (Class I to IV) will be summarized at each time point by treatment group as a shift table from baseline to post-baseline time point. Percentages will be based on the number of subjects with both a baseline and post-baseline (at the specific time point) assessment. The treatment difference of physician's assessment of functional class at Week 6 will be tested using the two-sided Pearson's chi-squared test.

The incidence of death and hospitalization will be summarized by treatment group. Two-sided 95% CIs for the observed difference in clinical events: death and hospitalizations between the treatment groups will be presented based on a continuity-correct Z-statistic. A by-subjects listing of subjects who died due to any cause will be provided.

3.4.3 Exploratory Efficacy Endpoints

Echo measurements of RV size and TAPSE will be summarized using descriptive statistics by visit for each treatment group. A listing of echo variables will be provided.

In addition, individual subject responses in PCWP with exercise will be compared to the select hemodynamic variables and derived indices (at baseline) and echo measurements (at baseline) for correlations to individual subject responses in PCWP (at Week 6) if data permit.

3.5 Pharmacokinetic Assessment

The Pharmacokinetic (PK) analyses will be documented in a separate file and performed by another vendor and reported separately.

3.6 Safety Assessment

Safety data will be summarized by actual treatment received (and in total for selected analyses) in the double-blind phase based on the Safety Analysis Set.

3.6.1 Adverse Events

An AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. All AEs are collected from the time the informed consent is signed until the subject discontinues or until Week 6 (whichever is first). All AEs will be coded to system organ class and preferred term using MedDRA version 21.0. Treatment-emergent adverse events (TEAEs) are defined as AEs that start after the first dose of study drug (lead-in infusion).

TEAEs with onset during the lead-in phase and double-blind phase will be summarized separately. TEAEs that start on or after the lead-in infusion and prior to the first double-blind infusion will be attributed to the lead-in phase, while TEAEs that start on or after the first double-blind infusion will be attributed to the double-blind phase. All AEs that start prior to the lead-in infusion will be listed.

Adverse events of special interest include the following preferred terms: hypotension, atrial fibrillation, clinically significant arrhythmia, aborted resuscitated death, and stroke.

An overview of TEAEs will be provided including counts and percentages of subjects with the following:

- Any TEAEs (overall and by maximum severity)
- Any study drug related TEAEs (overall and by maximum severity)
- Any TEAEs of special interest (overall and by maximum severity)
- Any serious TEAEs (TESAEs)
- Any TEAEs leading to discontinuation of study drug
- Any TEAEs leading to discontinuation of study
- Any TEAEs leading to death

Counts and percentages of subjects will also be presented by system organ class and preferred term for each of the categories in the overview.

Listings will be presented specifically for SAEs and TEAEs leading to discontinuation of study drug.

3.6.2 Clinical Laboratory Tests

All laboratory data will be converted to standard units during the Data Management process.

Summary statistics will be provided for laboratory values as well as their changes from baseline for all laboratory parameters by treatment group.

Counts and percentages of subjects with any post-baseline observation that is below the lower limit of normal (<LLN) or above the upper limit of normal (>ULN) will be summarized for each laboratory parameter by treatment group and total.

3.6.3 Vital Signs

Vital signs parameters (weight, body mass index, blood pressure [systolic and diastolic blood pressure], heart rate, body temperature, and respiration rate) will be summarized using descriptive statistics for by visit for each treatment group. The changes from baseline will also be presented.

A listing of all vital signs will be provided by subject.

3.6.4 Electrocardiograms

Electrocardiogram (ECG) parameters (PR, QRS, QT, and RR) will be summarized using descriptive statistics at baseline and Week 6/Early Termination for the treatment group. The overall interpretation will be summarized by counts and percentages by treatment group and total.

All ECG measurements and the overall interpretation will be listed by subject.

3.6.5 Cardiac Monitoring

Selected cardiac monitoring parameters will be summarized using descriptive statistics at baseline and Week 5 for the treatment group. Shifts from baseline to Week 5 will be presented for the clinical alert findings by treatment group and total.

All Cardiac monitoring data will be listed by subject.

3.6.6 Other Safety Parameters

The pregnancy test and Child-Pugh classification will be listed by subject and visit.

4 INTERIM ANALYSIS

No interim analysis is planned for this study.

5 CHANGES FROM PROTOCOL-SPECIFIED STATISTICAL ANALYSES

The protocol states that the hemodynamic variables will be analyzed using an ANCOVA model with effects for treatment and investigative site. The model name is corrected to be ANOVA model in the SAP.

The protocol states that one of the secondary endpoints is "Exercise duration 6-min walk test at Week 6". To clarify the endpoint, this endpoint is updated to "6MWT at Week 6 (distance)" in the SAP.

The change in mPAP and RAP (at rest and with exercise, baseline vs Week 6) are added to planned analyses of efficacy data. Both measurements provide useful assessments of responses to study drug by the right heart. Neither hemodynamic measurement was included in the protocol defined endpoints.

The protocol states that the major protocol deviation "Did not receive all weekly infusions" is an exclusion criterion for Per-Protocol Set. This criterion is updated to "Missed more than one study drug infusion" due to the impact of the COVID-19 pandemic. Other major protocol deviations with minor changes are updated due to the same reason.

6 PROGRAMMING SPECIFICATIONS

Analyses will be performed using SAS® version 9.3 or higher. All available data will be presented in subject data listings which will be sorted by subject and visit date as applicable. Detailed Programming Specifications will be provided in a separate document.