STATISTICAL ANALYSIS PLAN

Protocol Title: An Open-Label Rollover Study of Levosimendan in Patients with Pulmonary Hypertension with Heart Failure and Preserved Left Ventricular Ejection Fraction (PHHFpEF)

NCT Number: NCT03624010

Document Approval: 10 August 2023

***Note:** There is an error in the version history table of the attached document. There is no version 1.9 as listed in the last row of the table. The last version, 1.0, was approved on 10 August 2023 and is noted in the header of every page as such. There is also an error in the referenced protocol version. Although version 5.0 was approved (internally only), it was not implemented. A Note to File has been added to the TMF document, which identifies the last protocol version implemented (TNX-LVO-05, version 4.1) included in this NCT03624010 documentation upload.

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Protocol Title:	An Open-Label Rollover Study of Levosimendan in Patients with Pulmonary Hypertension with Heart Failure and Preserved Left Ventricular Ejection Fraction (PH-HFpEF)
Protocol Number:	TNX-LVO-05
Protocol Version/Date:	5.0 / 10Feb2022
Investigational Product:	Oral Levosimendan
Sponsor:	Tenax Therapeutics, Inc. One Copley Parkway Suite 490 Morrisville, NC 27560 info@tenaxthera.com
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SIGNATURE PAGE

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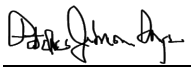
We, the undersigned, have reviewed and approved this Statistical Analysis Plan:

Signature		**Date**
	*Electronically signed by: Patches Johnson Inge* *Reason: Approved* *Date: Aug 15, 2023 11:47 EDT*	
Patches Johnson Inge, Ph.D. *Director, Biostatistics* *Medpace, Inc.*		
	*Electronically signed by: Stuart Rich* *Reason: Approved* *Date: Aug 14, 2023 19:03 CDT*	
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Douglas Hay, PhD *SVP, Regulatory Affairs* *Tenax Therapeutics, Inc.*		

VERSION HISTORY

Version	Version Date	Description
0.1	13Feb2023	Initial draft version
0.2	05Apr2023	Second draft after sponsor comments.
0.3	14Jul2023	Updated version according to comments and data
1.9	10Aug2023	Final version.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Abbreviation	Definition
6MWT	Six-minute Walk Test
AE	Adverse event
ATC	Anatomical therapeutic chemical
BID	Twice daily
BMI	Body mass index
BP	Blood pressure
BUN	Blood urea nitrogen
CABG	Coronary artery bypass graft
CRF	Case Report Form
CSR	Clinical Study Report
ECG	Electrocardiogram
IV	Intravenous
KCCQ	Kansas City Cardiomyopathy Questionnaire
MedDRA	Medical Dictionary for Regulatory Activities
NYHA	New York Heart Association
PH-HFpEF	Pulmonary Hypertension with Heart Failure and Preserved Left Ventricular Ejection Fraction
PK	Pharmacokinetic
PP	Per-Protocol
PR	Pulse rate
QID	Four times daily
QOL	Quality of life
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAS®	Statistical Analysis Software®
TEAE	Treatment-emergent adverse event
TFL	Tables, Figures and Listings
TID	Three times daily
WHO	World Health Organization

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-05. 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

The study objective is to continue treatment of levosimendan in patients who were participating in a Tenax-sponsored study after completion of the parent study. This open-label rollover study is designed to allow patients to continue levosimendan treatment if the patient has tolerated treatment in a clinical study sponsored by Tenax Therapeutics Inc. (the parent study) and if, in the opinion of the Investigator, the patient may benefit from continued levosimendan treatment. Patient safety will be evaluated in accordance with institutional standards of care.

2.1.1 Primary Objectives

The primary objectives are to evaluate the safety and tolerability of levosimendan in extended use after completion of the parent study.

2.1.2 Secondary Objective

The secondary efficacy objective is to evaluate whether the effectiveness of daily oral levosimendan is sustained in extended use.

2.1.3 Exploratory Objectives

No exploratory objectives are itemized for the study.

2.1.4 Pharmacokinetic (PK) Objectives

There are no PK objectives for this study.

2.2 Study Design

2.2.1 Overview

This extension study is designed to investigate the safety and efficacy of extended levosimendan in patients who previously received levosimendan in the Pulmonary Hypertension with Heart Failure and Preserved Left Ventricular Ejection Fraction (PH-HFpEF) study sponsored by Tenax and may, in the investigator's opinion, derive benefit from continued treatment. Patients will be screened for eligibility at the last visit in the treatment period of the parent study. Eligible patients will be enrolled into the study immediately upon confirmation of eligibility so that levosimendan is not interrupted.

Efficacy assessments (Six-minute walk test (6MWT), Patient global assessment (based on the Kansas City Cardiomyopathy Questionnaire (KCCQ) instrument), Physician's Assessment of Functional Class (measured by New York Heart Association (NYHA) functional class assessment), Clinical Events (death and hospitalizations)) will be performed at 3, 6, 12, 24, 48 weeks and yearly through the Follow-up/Termination visit. Patients will undergo a follow-up visit (at termination) as soon as possible after study drug discontinuation, but within one (1) week.

Patients whose blood pressure (BP) falls below the specified range will be queried regarding any symptoms of hypotension which may result in the down titration of the levosimendan dose. Patients whose heart rate exceeds the specified range will be queried regarding symptoms of worsening heart failure. An excessive heart rate in a patient with no evidence of worsening heart failure, suggests that the increased heart rate is a direct drug effect requiring adown titration of the levosimendan dose.

2.2.2 Randomization and Blinding

This is an open-label extension of the parent study. All patients who enroll into this study will receive an open-label levosimendan. No blinded treatment assignments are required.

2.2.3 Study Drug and Dosing Regimen

All patients entering the study will continue receiving intravenous (IV) levosimendan weekly, and some of them will be transitioned to an oral dose of levosimendan. Those currently receiving IV levosimendan weekly will return to the investigational site and receive 1mg levosimendan capsule daily for two weeks.

Patients may then be titrated up every 2 weeks at 1mg levosimendan increments up to maximum of 4 mg daily (1mg QID), based on assessments of safety and patient's clinical response. Patients who tolerate 3 mg (1mg TID) oral levosimendan TID that have not demonstrated a 6MWT equivalent or better than the recorded 6MWT at initiation of their IV to oral levosimendan may be titrated to 4 mg (1mg QID). Patients who experience identified safety concerns in should be down-titrated at 1 mg/day increments or discontinued at the physician's discretion.

2.2.4 Sample Size Determination

All patients completing the parent study and in the opinion of the Investigator, may benefit from continued treatment.

2.3 Study Endpoints

2.3.1 Primary Endpoints

The primary endpoints are the incidence and severity of adverse events (AEs) and the incidence and severity of abnormal clinical laboratory test results (hematology, chemistry), physical examinations, vital signs ([BP] and pulse rate [PR]) and 12-lead electrocardiogram (ECG) values.

2.3.2 Secondary Endpoints

The secondary efficacy endpoints of the study are measures assessed at Weeks 3, 6, 12, 24 and 48, and yearly through the follow-up/termination visit:

- 6-minute walk test (6MWT),
- Patient global assessment (based on the Kansas City Cardiomyopathy Questionnaire
- (KCCQ) instrument),
- Physician's Assessment of Functional Class (measured by the NYHA functional class assessment), and
- Clinical Events: Death and hospitalizations.

2.3.3 Exploratory Efficacy Endpoints

There are no exploratory efficacy endpoints.

2.3.4 Pharmacokinetic (PK) Endpoints

There are no PK endpoints for this study.

3 STATISTICAL METHODOLOGY

3.1 General Considerations

3.1.1 Analysis Day

Analysis day will be calculated from the date of first dose of study drug (first IV dose or first oral dose, whichever occurs first). 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 Analysis Visits

Scheduled visits will be assigned to analysis visits as recorded on the case report form (CRF).

3.1.3 Definition of Baseline

Baseline is defined as the last non-missing measurement prior to the first dose of study drug.

3.1.4 Summary Statistics

Categorical data will generally be summarized with counts and percentages of patients. 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 Hypothesis Testing

No hypothesis testing will be conducted for this study.

3.1.6 Evaluation of Site Effect

No evaluation of site-specific data for this study.

3.2 Analysis Populations

3.2.1 Safety Analysis Set (SAF)

The Safety Analysis Set (SAF) will consist of all patients who receive at least 1 dose of study drug. Patients will be analyzed according to dose received, summarized by IV dosing period, oral dosing period, and in total. The SAF will be used for the analysis of safety data.

3.3 Patient Data and Study Conduct

3.3.1 Patient Disposition

Counts and percentages of patients who were transitioned to oral dose, discontinued early from the study, and completed the study will be summarized by treatment/dose group and overall for the SAF. Reasons for early discontinuation will also be summarized. An associated patient-level listing will be provided.

3.3.2 Protocol Deviations

Protocol deviations will be identified based on the clinical data as defined in the Protocol Deviation Plan. The Protocol Deviation Plan will define all protocol deviations as either CSR reportable or non-CSR reportable deviations. Counts and percentages of participants with CSR reportable protocol deviations by deviation category will be summarized by treatment/dose group and overall for the SAF. A listing of CSR-reportable protocol deviations will be generated.

3.3.3 Demographic and Baseline Characteristics

Demographic and baseline characteristics outlined below will be summarized with descriptive statistics or counts and percentages of patients as appropriate by treatment/dose group and overall for the SAF.

- Age
- Sex
- Race
- Weight at baseline
- Height at baseline
- Body mass index (BMI) at baseline.

An associated patient-level listing will also be provided.

3.3.4 Concomitant Medications

Concomitant medications will be coded to anatomical therapeutic chemical (ATC) class and preferred term using the World Health Organization (WHO) Drug Dictionary Mar 2018 Global B3. For summary purposes, medications will be considered prior medications if they stopped prior to the first dose of study drug and concomitant medications if they were taken at any time after the first dose of study drug (i.e., started prior to the first dose of study drug and were ongoing or started after the first dose of study drug). Concomitant medications for IV dosing period are defined as medications that were taken any time after the first IV dose and prior to first oral dose. Concomitant medications for oral dosing period are defined as medications that were taken any time after the first oral dose. Based on this definition, the same concomitant medication can be taken in both IV dosing period and oral dosing period.

If a medication has incomplete start or stop dates, dates will be imputed to determine whether a medication should be considered prior or concomitant. Details on the imputation of incomplete dates will be provided in the final SAP to reflect CRF design and instructions.

Counts and percentages of patients taking concomitant medications by ATC class and preferred term will be summarized by IV dosing period, oral dosing period and in total based on the Safety Set. A patient-level listing of all concomitant medications will also be provided.

3.3.5 Study Drug Exposure

Days of exposure to study drug will be calculated as:

(date of last dose of study drug – date of first dose of study drug + 1).

Details regarding the last oral dose date imputation are as follows:

- For patients who filled out form Date of Visit and did not continue to next yearly visit or completed all yearly visits for oral study drug, last oral dose date will not be imputed. The last oral dose date recorded in the raw data will be used.
- For patients who completed the study or discontinued early from study prior to Jan 1, 2022, last oral dose date will not be imputed. The last oral dose date recorded in the raw data will be used.
- For patients who finished Date of Visit form at the follow-up visit, the date of follow-up (FU) visit will be compared to the date of oral doses recorded.
    - If date of FU visit > last oral dose date record + 1 year, then last oral dose date will be imputed as (last oral dose date recorded + 365).
    - If date of FU visit <= last oral dose date record + 1 year, then last oral dose date will be imputed as (FU visit date - 1).
- For all other patients, last oral dose date will be imputed as Feb 1, 2023.

Note that the exposure calculation is intended to describe the length of time a patient was exposed to study drug and therefore does not take study drug interruptions into account. Days of exposure to study drug will be summarized overall based on the SAF for both IV and oral administration.

Study drug exposure will be also presented in a patient-level listing.

3.4 Endpoint Assessment

The SAF will be the primary population for the efficacy analyses. Efficacy data will also be analyzed using the PP as supportive analyses for selected endpoints.

3.4.1 Primary Endpoints

The primary endpoints (Safety measures) are as follows:

- Incidence and severity of treatment-emergent adverse events (TEAEs),
- Incidence and severity of abnormal clinical laboratory test results (hematology, chemistry),
- Incidence of abnormal physical examination results,
- Incidence of abnormal vital sign values (blood pressure [BP] and pulse rate [PR]), and
- Incidence of abnormal 12-lead electrocardiogram (ECG) results or values.

3.4.2 Secondary Endpoints

The secondary endpoints (Efficacy measures) of the study are:

- 6-minute walk test score (6MWT),
- Patient global assessment score (based on the KCCQ instrument),
- Physician's Assessment of Functional Class (measured by NYHA Functional Class assessment), and
- Incidence of clinical events (death and hospitalizations).

All Secondary Endpoint measurements will be presented in patient-level listings.

3.4.3 Subgroup Analyses

No subgroup analyses will be performed for this study.

3.5 Primary Endpoint Assessment

Primary endpoint safety measures will be summarized by treatment/dose group and overall using descriptive statistics (number of observations, mean, median, standard deviation, minimum, maximum) for continuous variables and counts and percentages for categorical data. Summaries will be based on the SAF. Associated patient-level listings will also be provided for the primary endpoint safety measures. Primary endpoint efficacy measures will not be summarized but will be provided in patient-level listings.

3.5.1 Adverse Events (AEs)

Adverse events will be coded according to the MedDRA guidance version 21.0. An adverse event is considered treatment-emergent if the start date/time of the event is:

- On or after the date/time of first IV dose for IV dosing only patients and patients who transitioned to oral dosing and were in IV period,
- On or after the date/time of first oral dose for patients who transitioned to oral dosing and were in oral period.

An overview of AEs will be provided including counts and percentages of patients with the following:

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

Counts and percentages of patients will also be presented by system organ class and preferred term for each of the categories in the overview. TEAEs and TESAEs will be also provided in patient-level listings.

If an adverse event has incomplete start or stop dates, dates will be imputed to determine whether the adverse event was treatment-emergent. Details on the imputation for incomplete dates will be provided in the final SAP to correspond to CRF design and instructions. Summaries will be performed by treatment/dose group.

3.5.2 Clinical Laboratory Tests

Values and the associated change from baseline for each laboratory parameter will be calculated at each scheduled visit. Values considered to be abnormal (as defined by normal ranges) will be flagged for identification. All clinically significant lab values (hematology, chemistry and coagulation) will be presented in patient-level listings.

3.5.3 Vital Signs

Vital sign measurements (systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse rate (PR)) will be presented in patient-level listings.

3.5.4 Electrocardiograms (ECGs)

Abnormal ECG measurements will be presented in patient-level listings.

3.5.5 Physical Examinations

A patient-level listing of the physical examination assessments will be provided.

4 CHANGES FROM PROTOCOL-SPECIFIED STATISTICAL ANALYSES

This SAP does not deviate from the statistical analysis described in v5.0 of the protocol. Any deviations from the protocol or SAP will be described in the CSR.

5 PROGRAMMING SPECIFICATIONS

Analyses will be performed using SAS® version 9.4 or higher. All available data will be presented in patient data listings which will be sorted by patient and visit date as applicable. Detailed Programming Specifications will be provided in a separate document.

LIST OF TABLES AND LISTINGS

Number	Title	Population
Table 14.1.1	Patient Disposition	SAF
14.1.2	Protocol Deviations	SAF
14.1.3	Demographic and Baseline Characteristics	SAF
14.1.4.1	Study Drug Exposure – Intravenous	SAF
14.1.4.2	Study Drug Exposure - Oral	SAF
14.1.5	Concomitant Medications	SAF
14.3.1	Overview of Treatment-Emergent Adverse Events	SAF
14.3.2.1	Summary of Treatment-Emergent Adverse Events by System Organ Class and Preferred Term	SAF
14.3.2.2	Summary of Treatment-Emergent Adverse Events by System Organ Class, Preferred Term and Maximum Severity	SAF
14.3.2.3	Summary of Treatment-Emergent Adverse Events Related to Study Drug by System Organ Class and Preferred Term	SAF
14.3.2.4	Summary of Serious Treatment-Emergent Adverse Events by System Organ Class and Preferred Term	SAF
14.3.2.5	Summary of Treatment-Emergent Adverse Events Leading to Study Drug Discontinuation by System Organ Class and Preferred Term	SAF
14.3.2.6	Summary of Treatment-Emergent Adverse Events Leading to Study Discontinuation by System Organ Class and Preferred Term	SAF

Listing 16.2.1	Patient Disposition	All Patients
16.2.2	Protocol Deviations	All Patients
16.2.4.1	Demographic and Baseline Characteristics	All Patients
16.2.4.2	Study Drug Exposure	All Patients
16.2.4.3	Concomitant Medications	All Patients
16.2.6.1	Six-Minute Walk Test	All Patients
16.2.6.2	Patient Global Assessment (KCCQ Questionnaire)	All Patients
16.2.6.3	Physician's Assessment of Functional Class (NYHA)	All Patients
16.2.7.1	Treatment-Emergent Adverse Events	All Patients
16.2.7.2	Serious Treatment-Emergent Adverse Events	All Patients
16.2.8.1	Vital Signs	All Patients
16.2.8.2	Abnormal ECG Measurements	All Patients
16.2.8.3.1	Laboratory: Clinically Significant Chemistry	All Patients
16.2.8.3.2	Laboratory: Clinically Significant Hematology	All Patients
16.2.8.3.3	Laboratory: Clinically Significant Coagulation	All Patients
16.2.8.4	Physical Examination	All Patients