Statistical Analysis Plan

A Monocentric, Open-Label, Proof of Concept Study to Evaluate the Safety and Efficacy of NTZ at 500mg Twice Daily on Collagen Turnover in Plasma in NASH Patients with Fibrosis Stage 2 or 3.

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MEDPACE

STATISTICAL ANALYSIS PLAN

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Protocol Number:	NTZ-218-1
Current Protocol:	02-MAR-2019
Principal Investigator and Sponsor:	[redacted] Pinnacle Clinical Research 5109 Medical Drive, Suite 200 San Antonio, TX 78229
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SIGNATURE PAGE

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

Signature	Date
[redacted] Project Biostatistician Medpace, Inc.	03 MAY 2019 DD-MMM-YYYY
[redacted] Medical Monitor Medpace, Inc.	02-MAY-2019 DD-MMM-YYYY
[redacted] Principal Investigator and Sponsor Pinnacle Clinical Research	01 MAY 2019 DD-MMM-YYYY

VERSION HISTORY

Version	Version Date	Description
1.0	30-APR-2019	Original signed version

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ADaM	Analysis Data Model
AE	Adverse event
ALT	Alanine Aminotransferase
AST	Aspartate aminotransferase
ATC	Anatomical therapeutic chemical
BID	Twice daily
BMI	Body mass index
CI	Confidence interval
CK	Creatine Kinase
CRF	Case report form
CSR	Clinical Study Report
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
EOS	End of study
FSR	Fractional Synthesis Rate
MedDRA	Medical Dictionary for Regulatory Activities
MRE	Magnetic Resonance Elastrography
MRI	Magnetic resonance imaging
NAFLD	Non alcoholic fatty liver disease
NASH	Non alcoholic steatohepatitis
Q1	First quartile
Q3	Third quartile
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SDTM	Study Data Tabulation Model
TEAE	Treatment-emergent adverse event
TFL	Table, figure, and listing
ULN	Upper limit of normal
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 NTZ-218-1. 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 and Endpoints

Primary	
Objective	**Endpoint**
To evaluate the safety and tolerability of NTZ 500 mg twice daily (BID) after 24 weeks of treatment in patients with non alcoholic steatohepatitis (NASH)-induced stage 2 or stage 3 fibrosis.	Adverse events (AEs) including treatment-emergent AEs (TEAEs) and serious AEs (SAEs), laboratory evaluations, vital signs, electrocardiograms (ECGs), and physical examinations.
Secondary	
Objective	**Endpoint**
To determine the effect of oral administration of NTZ 500 mg BID on de novo collagen synthesis through Fractional Synthesis Rate (FSR) of circulating plasma proteins in patients with NASH induced stage 2 or stage 3 fibrosis.	Percent change in FSR from baseline to end of treatment (24 weeks of treatment).
To assess changes in liver stiffness as measured by FibroScan® and by Magnetic Resonance Elastography (MRE).	Observed change in liver stiffness by FibroScan from baseline to 24 weeks of treatment Observed change in liver stiffness by MRE from baseline to 12 weeks and 24 weeks of treatment.
To assess the change from baseline in non-invasive markers of fibrosis: ELF™ test score, PIIINP, Hyaluronic acid, CK18, TIMP-1, YKL-40, Alpha 2 macroglobulin, miRNA, ProC3, ProC6, FGF19, and FGF21.	Observed change in non-invasive biomarkers of fibrosis from baseline to 12 weeks and 24 weeks of treatment.
To assess changes in additional non-invasive tests: fibrosis scores: non alcoholic fatty liver disease (NAFLD) fibrosis score and FIB-4.	Observed change in fibrosis scores from baseline to 12 weeks and 24 weeks of treatment.

2.2 Study Design

2.2.1 Overview

This is a Phase 2 proof of concept study to evaluate the safety and efficacy of open-label NTZ 500 mg BID in a NASH population with NASH induced Stage 2 or 3 fibrosis.

The study will include a screening visit within 6 weeks of randomization (i.e. Day 1) followed by a 24-week treatment period consisting of daily, BID, oral administration of NTZ and an end of treatment follow up period of 4 weeks.

Patients will come to the research center and complete the following visits:

- Screening Visit within 6 weeks of Randomization (i.e. Day 1)
- Day -14 Deuterated Water Run-In
    - Day-14, Day-11, Day-7 and Day 1: sampling for labeling of Deuterated water during run-in,
- Repeat baseline labs for alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, alkaline phosphatase, creatine kinase (CK) collected at least 4 weeks from initial screening.
- Day 1 randomization: start of treatment
- Every 4 weeks after randomization until 20 weeks post randomization
- 22 weeks post enrollment and Deuterated Water Administration Period
    - 22 weeks plus 3 days
    - Sampling for labeling of Deuterated Water on Day 155, Day 158, Day 162, and Day 169
- 24 weeks post randomization: End of Treatment
- Week Post Treatment Follow Up

2.2.2 Randomization and Blinding

There is only one treatment group; there is no randomization. Any use of “randomization” refers to enrollment Day 1 (i.e. subject passed screening and will enter the treatment period). This is an open-label study; there is no blinding.

2.2.3 Study Drug

Patients will receive 500 mg NTZ (ALINIA®) twice daily for 24 weeks. NTZ will be supplied as 500 mg of ALINIA (nitazoxanide) tablets, for oral use. The tables are round, yellow, film-coated and debossed with ALINIA on one side and 500 on the other side. Each tablet contains 500 mg of nitazoxanide.

2.2.4 Sample Size Determination

The sample size of this Phase 2 proof of concept study is based on clinical assessment. Twenty male and female subjects with histologically confirmed NASH and fibrosis stage 2 or 3 will

provide sufficient information to assess the safety and tolerability of NTZ 500mg BID over 24 weeks.

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. 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). Where applicable, early termination visits will be summarized with Visit 10 Week 24 (i.e. Visit 10 Week 24/Early Termination).

3.1.3 Definition of Baseline

Baseline is defined as the last measurement prior to the first dose of study drug (generally should be the Day 1 pre-dose assessment).

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 in the table. Continuous data will generally be summarized with descriptive statistics including n (number of non-missing values), mean, standard deviation, first quartile (Q1), median, third quartile (Q3), minimum, and maximum.

3.1.5 Hypothesis Testing

This is a proof of concept study to assess the safety and tolerability of NTZ 500 mg BID over 24 weeks. There will be no formal hypothesis testing.

3.1.6 Handling of Dropouts and Missing Data

All data will be analyzed as observed. Dropouts and missing data will not be imputed.

3.2 Analysis Populations

3.2.1 Full Analysis Set

The Full Analysis Set consists of all subjects that meet the eligibility criteria and enroll into the study (Visit 1 Day 1).

The Full Analysis Set will be the primary population for all efficacy analyses. Only subjects with results for the specified efficacy parameter will be included in each analysis.

3.2.2 Safety Analysis Set

The Safety Analysis Set consists of all enrolled subjects who receive at least one dose of study drug.

The Safety Analysis Set will be the primary population for all safety analyses.

3.3 Subject Data and Study Conduct

3.3.1 Subject Disposition and Analysis Populations

Counts and percentages of subjects who were screened (signed informed consent), discontinued early during screening (screen failures), and enrolled (Full Analysis Set) will be summarized in total based on all screened subjects. Reasons for early discontinuation will also be summarized.

Counts and percentages of subjects in the Full Analysis Set, Safety Analysis Set, discontinued early from the study, and completed the study will be summarized based on the Full Analysis Set. Reasons for early discontinuation will also be summarized.

3.3.2 Protocol Deviations

Protocol deviations will be logged in the Medpace ClinTrak system. All protocol deviations will be listed.

3.3.3 Demographic and Baseline Characteristics

The following demographic and baseline characteristics will be summarized based on the Full Analysis Set:

- Age (years) at informed consent and age categories (<65 years, ≥65 years),
- Sex,
- Race,
- Ethnicity,
- Height (cm) at Screening,
- Baseline weight (kg), and
- Baseline body mass index (BMI) (kg/m^2).

3.3.4 Medical History

All medical history will be listed.

3.3.5 Concomitant Medications

Prior and concomitant medications will be coded to anatomical therapeutic chemical (ATC) class and preferred term using the World Health Organization (WHO) Drug Dictionary version Global B3 September 2018. 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 at the time of the first dose of study drug, or started after the first dose of study drug). Medications cannot be counted as both prior and concomitant.

If a medication has incomplete start or stop dates, dates will not be imputed to determine whether a medication should be considered prior or concomitant. If the date is missing but it is known that the medication was taken within the same month as the first dose of study drug, it will be considered concomitant. If the date and month are missing but it is known that the medication was taken within the same year as the first dose of study drug, it will be considered concomitant.

Counts and percentages of subjects taking prior and concomitant medications by ATC class and preferred term will be summarized based on the Safety Analysis Set.

3.3.6 Study Drug Exposure and Compliance

Days of exposure to study drug will be calculated as date of last dose of study drug as recorded on the end of study form – date of first dose of study drug + 1. Note that the exposure calculation is intended to describe the length of time a subject was exposed to study drug and therefore does not take study drug interruptions into account. Days of exposure to study drug will be summarized based on the Safety Analysis Set with descriptive statistics and with counts and percentages of subjects with exposure in the following categories:

- <4 weeks (<29 days)
- 4 to <8 weeks (29 – 56 days)
- 8 to <12 weeks (57 – 84 days)
- 12 to <16 weeks (85 – 112 days)
- 16 to <20 weeks (113 – 140 days)
- 20 to <24 weeks (141 – 168 days)
- ≥24 weeks (≥169 days)

Percent compliance to the study drug regimen will be calculated as 100 x number of actual capsules taken / number of expected capsules taken. The number of actual capsules taken will be calculated as number of capsules dispensed – number of capsules returned. If study drug is not returned, the number of capsules returned will be considered 0 for the compliance calculation, i.e. it will be assumed that the subject took all capsules. The number of capsules expected will be calculated as 2 x days of exposure. Percent compliance to the study drug regimen will be summarized based on the Safety Analysis Set with descriptive statistics and with counts and percentages of subjects with compliance in the following categories:

- <80%
- 80-120%
- >120%

3.4 Efficacy Assessments

3.4.1 Fractional Synthesis Rate

FSR of circulating plasma proteins will be measured at baseline and after 24 weeks of treatment. The observed change and percent change in FSR from baseline to Visit 10 Week 24 between the first and second deuterated water loading periods will be assessed to determine the effects of NTZ 500 mg BID on de novo collagen synthesis in the Full Analysis Set.

Wilcoxon signed-rank test will be used for statistical inference. Summary statistics of change in FSR (observed change and percent change) from baseline in FSR will be reported and evaluated at a significance level of 0.05. The p-value from Wilcoxon signed rank test will be reported. Only subjects with FSR at both baseline and Visit 10 Week 24 will be included in analysis.Liver Stiffness

FibroScan

Summary statistics for liver stiffness as measured by FibroScan will be provided at baseline and Visit 10 Week 24/Early Termination, including the change from baseline, based on the Full Analysis Set.

Student's T-test will be used for statistical inference. Summary statistics of change in liver stiffness as measured by FibroScan from baseline in FSR will be reported and evaluated at a significance level of 0.05. The 95% confidence interval (CI) and p-value from Student's T-test will be reported. Only subjects with FibroScan at both baseline and Visit 10 Week 24/Early Termination will be included in analysis.

MRE

Summary statistics for liver stiffness as measured by MRE will be provided at baseline, Visit 4 Week 12, and Visit 10 Week 24/Early Termination, including the changes from baseline, based on the Full Analysis Set. Student's T-test will be used for statistical inference in the same manner as for FibroScan.

3.4.2 Non-invasive Markers of Fibrosis

Biomarkers of fibrosis include ELF test score, PIIINP, Hyaluronic acid, CK18, TIMP-1, YKL-40, Alpha 2 macroglobulin, miRNA, ProC3, ProC6, FGF19, and FGF21.

Summary statistics for biomarkers will be provided at baseline, Visit 4 Week 12, Visit 10 Week 24/Early Termination, and at end of study (EOS) Week 28, including the changes from baseline, based on the Full Analysis Set. Student's T-test will be used for statistical inference in the same manner as for FibroScan.

3.4.3 Fibrosis Scores

NAFLD Fibrosis Score

Summary statistics for NAFLD fibrosis score will be provided at baseline, Visit 4 Week 12, and Visit 10 Week 24/Early Termination, including changes from baseline, based on the Full Analysis Set. Student's T-test will be used for statistical inference in the same manner as for FibroScan.

FIB-4

Summary statistics for FIB-4 will be provided at baseline, Visit 4 Week 12, and Visit 10 Week 24/Early Termination, including changes from baseline, based on the Full Analysis Set. Student's T-test will be used for statistical inference in the same manner as for FibroScan.

3.4.4 Exploratory Analysis

Additional serum and plasma samples will be maintained for future analysis. Exploratory measures for magnetic resonance imaging (MRI)/MRE sequence development will be maintained for future analysis.

Exploratory scatter plots will be provided for FSR vs. ProC3, FSR vs. liver stiffness as measured by MRE, and FSR vs. ELF test score, including the Pearson Correlation Coefficients and p-values. Additional exploratory analyses may also be performed to evaluate the association of individual exploratory biomarkers or combination of biomarkers with clinical measurements and other risk factors.

3.5 Safety Assessments

3.5.1 Adverse Events

All AEs regardless of seriousness or relationship to study drug, including those occurring during the Screening Period, are to be recorded on the corresponding page(s) of the CRF and in the patient's medical record from the informed consent form signature until study end for each patient. All AEs will be coded to system organ class and preferred term using Medical Dictionary for Regulatory Activities (MedDRA) version 21.0.

TEAEs are defined as AEs that worsen in severity or relationship to study drug or commence on or after the time of start of first study drug administration. This may include AEs that start during follow-up after the last dose of study drug.

Study drug related AEs are defined as AEs that are possibly related or related.

An overview of AEs based on the Safety Analysis Set 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 SAEs
- Any study drug related SAEs
- Any deaths due to AEs
- Any AEs leading to withdrawal from study or study drug
- Any study drug related AEs leading to withdrawal from study or study drug

Counts and percentages of subjects will also be presented by system organ class and preferred term for TEAEs and study drug related TEAEs. In addition, summaries will be provided by system organ class, preferred term, and maximum severity.

Listings will be presented specifically for SAEs and TEAEs leading to withdrawal from study or study drug.

3.5.2 Clinical Laboratory Tests

Blood and urine samples for chemistry, hematology, coagulation, urinalysis, and other safety markers will be processed by a central laboratory. Values and observed changes from baseline will be summarized at each scheduled visit (Visit 2 Week 4, Visit 3 Week 8, Visit 4 Week 12, Visit 5 Week 16, Visit 6 Week 20, Visit 10 Week 24/Early Termination, and EOS Week 28) by laboratory test based on the Safety Analysis Set.

Shift tables from baseline to maximum post-baseline value (including consideration of unscheduled visits) will be presented for ALT and AST (≤1x upper limit of normal [ULN], >1xULN to ≤3xULN, >3xULN to ≤5xULN, >5xULN), CK (≤1xULN, >1xULN to ≤2.5xULN, >2.5xULN to ≤5xULN, >5xULN), and total bilirubin (≤1xULN, >1xULN to ≤1.5xULN, >1.5xULN to ≤2xULN, >2xULN).

3.5.3 Vital Signs

Values and observed changes from baseline will be summarized at baseline and each scheduled visit (Visit 4 Week 12, Visit 10 Week 24/Early Termination, and EOS Week 28) for vital signs (systolic blood pressure, diastolic blood pressure, and heart rate), weight, and BMI based on the Safety Analysis Set.

3.5.4 Electrocardiograms

Values at baseline and Visit 10 Week 24/Early Termination, including the observed change from baseline, for 12-lead ECG parameters (heart rate, PR interval, QRS duration, QT interval, QTc interval, QTcF interval, and RR interval) will be summarized based on the Safety Analysis Set. The overall interpretations (normal, abnormal not clinically significant, or abnormal clinically significant) will be summarized by visit.

3.5.5 Physical Examinations

Physical examination findings (normal, abnormal not clinically significant, or abnormal clinically significant) at baseline, Visit 4 Week 12, Visit 10 Week 24/Early Termination, and EOS Week 28 will be summarized by body system based on the Safety Analysis Set.

3.5.6 Other Safety Assessments

Other safety assessments such as pregnancy tests will be listed.

4 DATA SAFETY MONITORING BOARD

An independent Data Safety Monitoring Board (DSMB) composed of 3 liver experts will review the safety of patients enrolled in the trial. The DSMB will perform a safety data review every 3 months, the first review occurring 3 months after first patient randomized. A DSMB charter will define the role, responsibilities, rules, and tasks of the DSMB.

5 ANALYSIS TIMING

5.1 Interim Analysis

No interim analysis is planned.

5.2 Pre-Final Analysis

After the database is locked, the pre-final analysis will be generated. Pre-final tables, figures, and listings (TFLs) will be provided approximately 3 weeks after database lock.

5.3 Final Analysis

After all comments on the pre-final analysis have been resolved and the study analysis database is declared final, the final analysis will be generated. Final TFLs will be provided approximately 1 week after the study database is declared final. If there were no changes to the pre-final analysis or the study database, the pre-final TFLs may be considered final. In addition to TFLs, Study Data Tabulation Model (SDTM) data and Analysis Data Model (ADaM) data along with associated files will be provided.

6 CHANGES FROM PROTOCOL-SPECIFIED STATISTICAL ANALYSES

There are no changes from the protocol-specified statistical analysis.

7 PROGRAMMING SPECIFICATIONS

Analyses will be performed using SAS® version 9.4. 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.