Protocol C4491004

A PHASE 1, RANDOMIZED, OPEN-LABEL, SINGLE DOSE STUDY TO INVESTIGATE THE PHARMACOKINETICS, PHARMACODYNAMICS, SAFETY AND TOLERABILITY OF VUPANORSEN ADMINISTERED SUBCUTANEOUSLY TO HEALTHY CHINESE ADULTS

Statistical Analysis Plan
(SAP)

Version: 1

Date: 16 October 2020

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1. VERSION HISTORY

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
1 16 October 2020	Original 28 Aug 2020	N/A	N/A

2. INTRODUCTION

This statistical analysis plan (SAP) provides the detailed methodology for summary and statistical analyses of the data collected in Study C4491004. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition or its analysis will also be reflected in a protocol amendment.

2.1. Study Objectives, Endpoints, and Estimands

The followings are objectives and endpoints in this study. Estimand framework is not applied to this phase 1 study.

Objectives	Endpoints
Primary:	**Primary:**
• To characterize the PK of vupanorsen following 80 and 160 mg single subcutaneous dose in healthy Chinese adults.	• Vupanorsen plasma PK parameters: AUC_{24h}, AUC_{48h}, AUC_{last}, C_{max}, AUC_{inf}, T_{max}, $t_{½}$, CL/F and Vz/F, as data permit.
Secondary:	**Secondary:**
• To evaluate the safety and tolerability of vupanorsen following 80 and 160 mg single subcutaneous dose in healthy Chinese adults. • To characterize the PD effects of vupanorsen following 80 and 160 mg single subcutaneous dose in healthy Chinese adults.	• Adverse events, clinical safety laboratory tests (serum chemistry, hematology, urinalysis, coagulation), vital signs (BP, PR), physical examination findings, 12-lead ECGs. • Percentage change from baseline in ANGPTL3 on Day 2, Day 3, Day 8, Day 15, Day 30, Day 60 and Day 90. • Percentage changes from baseline in Lipid Panel on Day 2, Day3, Day 8, Day 15, Day 30, Day 60 and Day 90,

	including fasting TG, LDL-C, HDL-C, VLDL-C, non-HDL-C, and TC. • Percentage changes from baseline in ApoA-I, ApoB total (including ApoB-48, ApoB-100), and ApoC-III on Day 15, Day 60 and Day 90.

2.2. Study Design

This is a Phase 1, randomized, parallel-cohort, open-label study to characterize the PK, PD safety and tolerability of vupanorsen following 80 mg and 160 mg single subcutaneous dose in healthy Chinese adults with fasting triglyceride ≥90 mg/dL. Approximately 18 participants will be enrolled in this study (N=9 for each cohort) with the goal of attaining approximately 8 evaluable participants in each cohort for a total of 16 evaluable participants.

The total duration of participation in this study is approximately 118 days, including a screening period of up to 28 days to confirm eligibility and a follow-up period of approximately 90 days. Eligible participants will progress to admission to the clinical research unit on Day -1 for a 4-day (3-night) inpatient stay. Participants enrolled will receive a single 80 mg (cohort 1) or 160 mg (cohort 2) subcutaneous dose of vupanorsen on Day 1. Following discharge on Day 3, participants will return for an on-site post-treatment evaluation on Days 8, 15, 30, 60 and 90.

Participants who discontinue prior to completion of the study for non-safety related reasons may be replaced, at the discretion of the principal investigator and sponsor study team.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoint(s)

Primary endpoints are following vupanorsen plasma PK parameters:

Table 2. Plasma Vupanorsen Pharmacokinetic Parameters

Parameter	Definition	Method of Determination
Cmax	Maximum plasma concentration	Observed directly from data
Tmax	Time at which Cmax occurred	Observed directly from data as time of first occurrence
AUC24h	Area under the concentration-time profile from time 0 to 24 hour post-dose	Linear/log trapezoidal method
AUC48h	Area under the concentration-time profile from time 0 to 48 hour post-dose	Linear/log trapezoidal method

Table 2. Plasma Vupanorsen Pharmacokinetic Parameters

Parameter	Definition	Method of Determination
AUC_{last}	Area under the concentration-time profile from time 0 to the time of the last quantifiable concentration (C_{last})	Linear/log trapezoidal method
AUC_{inf}*	Area under the concentration-time profile from time 0 extrapolated to infinite time	AUC_{last} + (C_{last}*/kel), where C_{last}* was the predicted serum concentration at the last quantifiable time point estimated from the log-linear regression analysis
$t_{½}$*	Terminal elimination half-life	Loge(2)/kel, where kel was the terminal phase rate constant calculated by a linear regression of the log-linear concentration-time profile
CL/F*	Apparent clearance	Dose/AUC_{inf}
V_z/F*	Apparent volume of distribution	Dose/(AUC_{inf}*kel)

* If data permitted.
Pharmacokinetic parameters will be calculated using an internally validated software system, electronic non-compartmental analysis (eNCA, version 2.2.4 or higher).

3.2. Secondary Endpoint(s)

3.2.1. Safety Endpoints

Safety endpoints are:

- Adverse events (AE);
- Clinical safety laboratory measurements, including serum chemistry, hematology, urinalysis, and coagulation;
- Vital signs, including blood pressure (BP) and pulase rate (PR);
- Physical examination findings;
    - Any untoward findings identified on physical examinations conducted during the active collection period will be captured as AEs, if those findings meet the definition of an AE.
- 12-lead ECGs.

The details of the safety endpoints are described in Section 3.5.

3.2.2. Pharmacodynamics Endpoints

PD endpoints are:

- Percentage change from baseline in ANGPTL3 on Day 2, Day 3, Day 8, Day 15, Day 30, Day 60 and Day 90;
- Percentage changes from baseline in Lipid Panel on Day 2, Day3, Day 8, Day 15, Day 30, Day 60 and Day 90, including fasting TG, LDL-C, HDL-C, VLDL-C, non-HDL-C, and TC;
- Percentage changes from baseline in ApoA-I, ApoB total (including ApoB-48, ApoB-100), and ApoC-III on Day 15, Day 60 and Day 90.

3.3. Other Endpoint(s)

Not applicable.

3.4. Baseline Variables

There are no baseline variables to be used as covariates or stratification factors in this study.

The demographic data will include age, sex, weight, body mass index. Baseline variables are those collected on Day 1 prior to dosing or last measurement before Day 1.

3.5. Safety Endpoints

3.5.1. Adverse Events

All events that start on or after the first dosing day and time/start time, if collected, but before the last dose plus the lag time as defined in the protocol will be flagged as Treatment-Emergent Adverse Events (TEAEs). If an AE starts on the same day as the first dose date, it will be considered treatment emergent unless the case report form data indicates otherwise via explicitly recording time for AE onset and treatment dosing.

3.5.2. Laboratory Data

Safety laboratory tests will be performed as described in the protocol.

To determine if there are any clinically significant laboratory abnormalities, the hematological, clinical chemistry, urinalysis, and coagulation safety tests will be assessed against the criteria specified in the sponsor reporting standards. The assessment will not take into account whether each participant's baseline test result is within or outside the laboratory reference range for the particular laboratory parameters. Baseline will be the last predose measurement collected on Day -1.

3.5.3. Vital Signs

Supine blood pressure and pulse measurements will be taken at all time points listed in the Schedule of Activities given in the protocol. Baseline will be defined as the measurement obtained on Day 1 predose.

The maximum decrease and increase from baseline over all measurements taken postdose for supine systolic and diastolic blood pressures will be determined. The maximum increase from baseline will be calculated by first subtracting the baseline value from each postdose measurement to give the change from baseline. The maximum of these values over the entire study will then be selected, except in the case where a participant does not show an increase. In such an instance, the minimum decrease should be taken.

Similarly, the maximum decrease from baseline will be determined by selecting the minimum value of the change from baseline. In cases where a participant does not show a decrease, the minimum increase should be taken.

3.5.4. Electrocardiograms

Standard 12-lead ECGs will be taken at all time points listed in the Schedule of Activities given in the protocol. Baseline will be defined as the measurement obtained on Day 1 predose.

The QT, QTcF, PR, QRS and heart rate (HR) will be recorded at each assessment time.

If not supplied, QTcF will be derived using Fridericia's heart rate correction formula:

$QTcF = QT/(RR)^{1/3}$ where respiratory rate (RR) = 60/HR (if not provided)

The maximum absolute value (postdose) and the maximum increase from baseline will be determined over all measurements taken postdose for QTcF, PR and QRS.

The maximum increase from baseline will be calculated by first subtracting the baseline value from each postdose measurement to give the change from baseline. The maximum of these values over the entire study will then be selected, except in the case where a participant does not show an increase. In such an instance, the minimum decrease should be taken.

4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

For purposes of analysis, the following analysis sets are defined:

Participant Analysis Set	Description
Enrolled	All participants who sign the informed consent document (ICD).
Pharmacokinetics (PK)	The PK concentration population is defined as all participants enrolled and treated who have at least 1 vupanorsen concentration. The PK parameter analysis population is defined as all participants enrolled and treated who have at least 1 of the vupanorsen PK parameters of interest.
Pharmacodynamics (PD)	The PD parameter analysis population is defined as all participants enrolled and treated who have at least 1 of the PD parameters of interest.
Safety	All participants enrolled and who take at least 1 dose of study intervention.

If a participant receives a treatment that is not consistent with the treatment they are assigned to, then the participant will be reported under the treatment that the participant actually received for all PK, PD and safety analyses, where applicable.

5. GENERAL METHODOLOGY AND CONVENTIONS

The study will be analyzed and reported once all participants have completed the study (or discontinued), ie, following the data set release.

5.1. Hypotheses and Decision Rules

There are no statistical hypotheses or decision rules.

5.2. General Methods

All data will be descriptively summarized by treatment (vupanorsen 80 mg and 160 mg).

5.2.1. Analyses for Binary Endpoints

Binary endpoints will be summarized by treatment and visit (if appropriate) with number and percentage of participants in each category, if not otherwise specified.

5.2.2. Analyses for Continuous Endpoints

Continuous endpoints will be summarized by treatment and visit using descriptive statistics, including n, arithmetic mean, median, standard deviation (SD), minimum and maximum, if not otherwise specified.

5.2.3. Analyses for Categorical Endpoints

Categorical endpoints will be summarized by treatment and visit (if appropriate) with number and percent of participants in the treatment group within each category, if not otherwise specified.

5.3. Methods to Manage Missing Data

5.3.1. Safety Data

For the analysis of safety endpoints, the sponsor data standard rules for imputation will be applied.

5.3.2. Pharmacokinetic Data

Concentrations Below the Limit of Quantification

In all data presentations (except listings), concentrations below the limit of quantification (BLQ) will be set to zero. (In listings BLQ values will be reported as "<LLQ", where LLQ will be replaced with the value for the lower limit of quantification.)

Deviations, Missing Concentrations and Anomalous Values

In summary tables, plots of mean profiles and plots of median profiles, summary statistics will be calculated setting concentrations to missing if one of the following cases is true:

1. A concentration has been collected as ND (ie, not done) or NS (ie, no sample);

2. A deviation in sampling time is of sufficient concern or a concentration has been flagged anomalous by the pharmacokineticist.

Note that summary statistics will not be presented at a particular time point if more than 50% of the data are missing.

Pharmacokinetic Parameters

Actual PK sampling times will be used in the derivation of PK parameters.

If a PK parameter cannot be derived from a participant's concentration data, the parameter will be coded as NC (ie, not calculated). (Note that NC values will not be generated beyond the day that a participant discontinues.)

In summary tables, statistics will be calculated by setting NC values to missing; and statistics will be presented for a particular dose with ≥3 evaluable measurements.

If an individual participant has a known biased estimate of a PK parameter (eg, due to known loss of drug during subcutaneous administration), this will be footnoted in summary tables and will not be included in the calculation of summary statistics.

5.3.3. Pharmacodynamic Data

Missing values will not be imputed.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoint(s)

To assess the pharmacokinetics of vupanorsen, the PK parameters detailed in Section 3.1 will be listed, summarized and plotted by treatment (vupanorsen 80 mg and 160 mg) for participants in the PK parameter analysis set (as defined in Section 4). Missing values will be handled as detailed in Section 5.3.2. Each PK parameter will be summarized by treatment and will include the set of summary statistics as specified in the table below:

Table 3. PK Parameters to be Summarized Decsriptively by Treatment

Parameter	Summary Statistics
C_{max}, AUC_{24h}, AUC_{48h}, AUC_{last}, AUC_{inf}, CL/F, V_z/F	n, arithmetic mean, median, SD, minimum, maximum, percent coefficient of variation (%CV), geometric mean and geometric %CV
T_{max}	n, median, minimum, maximum
$t_{½}$	n, arithmetic mean, median, %CV, SD, minimum, maximum

There will be one summary table presenting all PK parameters.

To assess the relationship between the PK parameters and dose, box and whisker plot of dose normalized C_{max}, AUC_{24h}, AUC_{48h}, AUC_{last}, and AUC_{inf} will be plotted against dose, and will include individual participant values and geometric means for each dose. Geometric means will have a different symbol than individual values. The values will be dose normalized (to 1 mg dose) by dividing the individual values and raw geometric means by dose. A footnote will be added to the plots to indicate that geometric means are presented.

The PK concentrations will be listed and summarized for participants in the PK concentration analysis set. Presentations for PK concentrations will include:

- A listing of all concentrations sorted by subject ID, treatment and nominal time postdose. The concentration listing will also include the actual times. Deviations from the nominal time will be given in a separate listing. The listing will be paged by treatment.

- A summary of concentrations by treatment and nominal time postdose, where the set of statistics will include n, mean, median, standard deviation, coefficient of variation (cv), minimum, maximum and the number of concentrations above the lower limit of quantification.

- Median concentrations time plots (on both linear and semi-log scales) against nominal time post dose by treatment (all treatments on the same plot per scale, based on the summary of concentrations by treatment and time postdose).

- Mean concentrations time plots (on both linear and semi-log scales) against nominal time post dose by treatment (all treatments on the same plot per scale, based on the summary of concentrations by treatment and time postdose).

- Individual concentration time plots by treatment (on both linear and semi-log scales) against actual time postdose. There will be separate spaghetti plots for each treatment per scale.

The length of time used for the x-axes of these plots will be decided on review of the data and will depend on how long vupanorsen concentration is quantifiable in the matrix.

For summary statistics, median and mean plots by sampling time, the nominal PK sampling time will be used, for individual subject plots by time, the actual PK sampling time will be used. For pre-dose, the actual PK sampling time will be set to 0 hour.

6.2. Secondary Endpoint(s)

6.2.1. Safety Endpoints

The details of safety analyses are described in the Section 6.6.

6.2.2. Pharmacodynamic Endpoints

The PD analysis will be conducted in the PD analysis set (as defined in Section 4). Non-fasting results will not be included in the analysis.

The baseline, the actual values, changes and percent changes from baseline in the following endpoints will be summarized by treatment and visit using descriptive statistics.

- ANGPTL3, TC, LDL-C, HDL-C, non-HDL-C, VLDL-C, TG, ApoA-I, ApoB total (including ApoB-48, ApoB-100), and ApoC-III.

Additionally, the following graphical summaries will be provided for the actual values, changes and the percent changes from baseline over time:

- Mean ± SD by treatment and day (all treatments on the same plot);
- Individual plots against day (there will be separate plots for each treatment).

A listing for the actual values, changes and percent changes from baseline with fasting status sorted by treatment, subject ID and day will be presented.

6.3. Other Endpoint(s)

Not applicable.

6.4. Subset Analyses

Not applicable.

6.5. Baseline and Other Summaries and Analyses

6.5.1. Baseline Summaries

Demographic and baseline characteristics (age, gender, weight, height and body mass index) collected prior to the first dose of the study intervention will be summarized following the CaPS.

6.5.2. Study Conduct and Participant Disposition

The following participant dispositions will be reported following the sponsor reporting standards:

- A summary of participant discontinuations up to the end of study;
- Summary of participant dispositions analyzed for PK and PD, as well as for safety by treatment group;
- Summary of numbers of participant treated by treatment group.

6.5.3. Concomitant Medications and Nondrug Treatments

All concomitant medications as well as nondrug treatments will be provided in the listings.

6.6. Safety Summaries and Analyses

The safety endpoints are presented in the Section 3.5. A set of summary tables split by treatment will be produced to evaluate any potential risk associated with the safety and toleration of administering vupanorsen.

No formal analyses are planned for safety data. The safety endpoints will be listed and summarized in accordance with the sponsor reporting standards, where the resulting data presentations will consist of participants from the safety analysis set.

6.6.1. Adverse Events

Adverse events will be listed and summarized by treatment in accordance with the sponsor reporting standards.

6.6.2. Laboratory Data

Laboratory data will be listed and summarized by treatment in accordance with the sponsor reporting standards. Baseline is as defined in Section 3.5.2.

Incidence of laboratory test abnormalities (without regard to baseline abnormality) will be summarized by treatment based on CaPS.

6.6.3. Vital Signs

Absolute values and changes from baseline in supine systolic and diastolic blood pressure and pulse rate will be summarized by treatment and postdose timepoints, according to the sponsor reporting standards. Tables will be paged by parameter. Baseline is as defined in Section 3.5.3.

Maximum increase or decrease in vital signs will also be summarized; all planned and unplanned postdose timepoints will be included in these summaries.

6.6.4. Electrocardiograms

Absolute values and changes from baseline in QT, heart rate, QTcF, PR and QRS will be summarized by treatment and postdose timepoints following the sponsor reporting standards. Tables will be paged by parameter. Baseline is as defined in Section 3.5.4.

The number (%) of participants with maximum postdose QTc values and maximum increases from baseline in the following categories will be tabulated by treatment:

Safety QTc Assessment

Degree of Prolongation	Mild (msec)	Moderate (msec)	Severe (msec)
Absolute value	>450-480	>480-500	>500
Increase from baseline		30-60	>60

In addition, the number of participants with uncorrected QT values >500 msec will be summarized.

Listings of participants with any single postdose value ≥500 msec will also be produced for QTcF.

7. INTERIM ANALYSES

No formal interim analysis will be conducted for this study. As this is an open-label study, the sponsor may conduct unblinded reviews of the data during the course of the study for the purpose of safety assessment.

8. REFERENCES

None.

9. APPENDICES

Appendix 1. List of Abbreviations

Abbreviation	Term
AE	adverse event
ANGPTL3	angiopoietin-like protein 3
ApoA-I	apolipoprotein A-1
ApoB	apolipoprotein B
ApoC-III	apolipoprotein C-III
AUC	area under the concentration-time curve
AUC_{24h}	AUC from time 0 to 24 hours post-dose
AUC_{48h}	AUC from time 0 to 48 hours post-dose
AUC_{inf}	AUC from time 0 extrapolated to infinite time
AUC_{last}	AUC from time 0 to T_{last}
BLQ	below the limit of quantification
BP	blood pressure
CaPS	CDISC and Pfizer Standard
CDISC	clinical data interchange standards consortium
CL/F	apparent clearance
C_{max}	maximum observed concentration
CV	coefficient of variation
ECG	electrocardiogram
HDL-C	high density lipoprotein cholesterol
HR	heart rate
ICD	informed consent document
ID	identification
LDL-C	low density lipoprotein cholesterol
LLQ	lower limit of quantification
N/A	not applicable
NC	not calculated
ND	not done
Non-HDL-C	non-high-density lipoprotein cholesterol
NS	no sample
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PR	pulse rate
QTc	corrected QT
QTcF	corrected QT (Fridericia method)
RR	respiratory rate
SAP	statistical analysis plan
SD	standard deviation
$t_{½}$	terminal elimination half life
TEAE	treatment-emergent adverse event
TC	total cholesterol

Abbreviation	Term
TG	triglyceride
T_{max}	time at which Cmax occurred
VLDL-C	very low density lipoprotein cholesterol
V_z/F	apparent volume of distribution

Appendix 2. Categorical Classes for Vital Signs

Systolic BP (mm Hg)	min. <90	
Systolic BP (mm Hg) change from baseline	max. decrease ≥30	max. increase ≥30
Diastolic BP (mm Hg)	min. <50	
Diastolic BP (mm Hg) change from baseline	max. decrease ≥20	max. increase ≥20
Seated pulse rate (bpm)	min. <40	max. >120

Measurements that fulfill these criteria are to be listed in report.