Protocol C5301022

A Pharmacokinetic Study of Zavegepant Intranasal in Healthy Adults Comparing Conventional Venous Blood Sampling with Patient-Centric Sampling

Statistical Analysis Plan
(SAP)

Version: 2

Date: 09 Sep 2023

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NOTE: *Italicized* text within this document has been taken verbatim from the Protocol.

1. VERSION HISTORY

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
1 / 14 Jul 2023	Amendment 1 12 Jun 2023	N/A	N/A
2 / 09 Sep 2023	Amendment 1 12 Jun 2023	To correct by-group displays and to add comparison of PK parameters between groups	Clarified that B/P ratio adjustment is only for Tasso-M20 in Sections 3.1.1, 3.1.2 and 6.1.1. Updated safety analyses to present by Tasso devise used and by race in Sections 6.5, 6.5.1 and 6.5.2. Removed by treatment analysis in Sections 3.5.1, 3.5.2 and 6.4.2. Updated PK analyses to present by collection method and by race in Sections 6.1.1 and 6.1.2. Added derivation of adjusted GMR for comparison of collection methods and race in Section 6.1.2.

2. INTRODUCTION

Zavegepant (PF-07930207) is a high affinity, selective, and structurally unique, small molecule calcitonin gene-related peptide (CGRP) receptor antagonist, which was recently approved by the Food and Drug Administration (FDA) for the acute treatment of migraine with or without aura in adults via intranasal (IN) administration. As a component of zavegepant clinical development, future pediatric studies will be conducted to evaluate the safety, tolerability, and pharmacokinetics (PK) of zavegepant in participants 6 to less than 12 years of age with migraine.

Recruiting and retaining pediatric patients in clinical trials has always been challenging due to a variety of factors, including inconvenience with patient preferences. To overcome some of those challenges, it is essential to prioritize patient-centric approaches that cater to the unique needs of pediatric patients. One such approach is patient-centric sampling (PCS), which aims to reduce the burden on pediatric patients by minimizing the volume of the biological samples required and being less invasive with minimal to no pain compared to the venous phlebotomy collection method, which is particularly beneficial for pediatrics who are fearful of needles. Additionally, PCS offers greater flexibility to pediatric patients and their families by allowing at-home sample collection for those who may have difficulty traveling to clinics.

To allow the use of PCS in zavegepant pediatric studies, a bridging or comparative study comparing the PCS approach with an equivalent conventional sampling technique is needed as a regulatory expectation to demonstrate the concordance of data from PCS in a controlled environment prior to implementation in large‐scale clinical trials. Therefore, the primary objective of this study is to establish the correlation between zavegepant concentration from samples collected using PCS devices compared to conventional venous blood sampling. In addition, if feasible, this study will also determine the PK and safety of zavegepant IN in Japanese participants.

Lastly, based on data from zavegepant IN clinical trials, dysgeusia (bad/metallic/bitter taste) has been reported as the most common treatment‐emergent adverse event with zavegepant. For instance, in a Phase 2/3, double-blind, randomized, placebo-controlled study (BHV3500-201), dysgeusia was reported as the most common TEAEs with zavegepant 10 and 20 mg IN and placebo (13.5% to 16.1% vs 3.5%, respectively). Similarly, results from a Phase 3 study of zavegepant IN administration for the treatment of acute migraine BHV3500-301 have reported dysgeusia as the most common TEAEs after zavegepant 10 mg IN administration with an incidence rate of 20.5% vs 4.7% in placebo. Because of this adverse event, patients may not adhere to their medications which may limit the effectiveness of treatment with zavegepant. Therefore, understanding this adverse event (eg, time-course, description of the taste sensation) and potential strategies to mask the unpleasant taste could help improve patient experience and adherence. As an exploratory objective, this study will evaluate palatability attributes of zavegepant and whether eating a butterscotch candy before zavegepant IN administration will help manage dysgeusia.

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in Study C5301022.

2.1. Modifications to the Analysis Plan Described in the Protocol

None.

2.2. Study Objectives, Endpoints, and Estimands

The following are the objectives and endpoints in this study. Estimand framework will not be applied to this Phase 1 study in healthy participants.

Objectives	***Endpoints***
Primary:	***Primary:***
• *Characterize the PK profile of zavegepant from samples collected using Tasso Devices (Tasso-Plus and Tasso-M20) vs. standard venous phlebotomy following IN administration of 10 mg of zavegepant IN formulation in healthy participants*	• *Zavegepant concentrations from samples collected using Tasso devices vs. standard venous phlebotomy* • *AUC_{last}, C_{max}, T_{max}, $t_{½}$, CL/F and V_z/F, as data permits*

Objectives	*Endpoints*
Secondary:	***Secondary:***
• *Evaluate the safety and tolerability following IN administration of 10 mg of zavegepant IN formulation in healthy participants*	• *Assessment of TEAEs, and clinical safety laboratory tests*
Exploratory:	***Exploratory:***
• *Evaluate the effect of the administration of 10 mg of zavegepant IN with a butterscotch candy on dysgeusia compared to zavegepant IN alone*	• *Taste Assessment Survey Scoring Metrics after study intervention: overall liking, bitterness, tongue/mouth burn, throat burn, sour taste, salty taste, and sweet taste*

2.3. Study Design

This is a single-center, Phase 1, non-randomized, open-label, 2-period study in healthy participants to primarily evaluate the correlation of zavegepant concentration from samples collected using Tasso Devices (Tasso-Plus and Tasso-M20) compared to standard venous phlebotomy. This study consists of two periods which will include approximately 14 participants.

In period 1, 50% (n=7) of participants will use Tasso-Plus, while the other 50% (n=7) will use Tasso-M20. All 14 participants will have 6 PK samples collected using the assigned Tasso device simultaneously with collecting venous blood samples at the following time points, 30 minutes, 1, 2-, 4-, 8-, and 12-hour postdose, as described in the SoA, Table 2 (in the protocol). In addition, taste assessments will be performed at time intervals of 1 (immediately after dosing), 5, 10 and 20 minutes after zavegepant IN administration. Also, if feasible, 4 Japanese participants will be enrolled among those 14 participants to evaluate the PK and safety of zavegepant IN in Japanese vs. non-Japanese participants. For Japanese participants, a total of 12 venous blood samples will be collected at the following time points, 0, 15 minutes, 30 minutes, 1, 1.5-, 2-, 3-, 4-, 6-, 8-, 12-, and 24-hour (refer to SoA, Table 2 in the protocol).

In period 2, a butterscotch candy will be given 5 minutes before administering the zavegepant IN study intervention. Taste assessment will also be performed after zavegepant IN administration with a butterscotch candy in period 2. For taste assessment, each participant will record the sensory attributes at timed intervals of 1 (immediately after dosing), 5, 10 and 20 minutes after zavegepant administration in each period.

Healthy participants will be screened within 28 days prior to the first administration of the study intervention to confirm that they meet the participant selection criteria for the study. Eligible participants will be admitted to the CRU on Day -1 and will be confined in the CRU until Discharge on Day 3 (SoA in the protocol), approximately 24 hours after the second dose. Enrolled participants will receive a single IN dose of zavegepant 10 mg (0.1 mL) in each period. PK venous blood samples as well as Tasso PK sampling, using Tasso-Plus and

Tasso-M20, will be collected at specified intervals as per SoA (Table 2) in the protocol for PK assessments.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoints

3.1.1. Zavegepant PK Concentrations

Venous blood and Tasso PK samples will be collected according to the SoA given in the protocol. For each individual, blood to plasma (B/P) ratios will be calculated by dividing concurrent (i.e. Day 1, 1 hour and 8 hour post-dose) whole blood zavegepant concentrations with the plasma zavegepant concentrations. The plasma equivalent zavegepant concentration by microsampling using the Tasso-M20 will be calculated by dividing with the individual B/P ratio. For the Tasso-Plus, normalization of the zavegepant concentration is not needed.

3.1.2. Zavegepant PK Parameters

Zavegepant PK parameters from samples collected using Tasso and venous phlebotomy will be derived from the concentration-time profile using non-compartmental methods, as detailed in Table 2. Actual PK sampling times will be used in the derivation of PK parameters. In the case that actual PK sampling times are not available, nominal PK sampling time will be used in the derivation of PK parameters.

Table 2. Definitions of PK Parameters

Parameter	***Definition***	***Method of Determination***
C_{max}	*Maximum plasma concentration*	*Observed directly from data*
AUC_{last}	*Area under the plasma concentration-time profile from time 0 to the time of the last quantifiable concentration (C_{last})*	*Linear/Log trapezoidal method*
AUC_{inf}[a]	*Area under the plasma concentration-time profile from time 0 extrapolated to infinite time*	*AUC_{last} + (C_{last}/k_{el}), where C_{last} is the predicted plasma concentration at the last quantifiable time point estimated from the log-linear regression analysis*
T_{max}	*Time for C_{max}*	*Observed directly from data as time of first occurrence*
$t_{1/2}$[a]	*Terminal half-life*	*$Log_e(2)/k_{el}$, where k_{el} is the terminal phase rate constant calculated by a linear regression of the log-linear concentration-time curve*
CL/F[a]	*Apparent clearance*	*Dose/AUC_{inf}*
V_z/F[a]	*Apparent volume of distribution*	*Dose/($AUC_{inf} \times k_{el}$)*

a. If data permits.

PK parameters from samples collected using Tasso-M20 will be adjusted for B/P ratio.

3.2. Secondary Endpoints

The secondary endpoints include the safety and tolerability of zavegepant as characterized by adverse events and clinical laboratory tests (discussed in Section 3.5).

3.3. Other Endpoint

The data collected for taste assessment using the sponsor-provided questionnaire will be numerically derived by measuring the length (using a scale with gradation of at least 0.1 centimeter) of the "x" marked by the participant relative to the "good trait." For taste assessment, the data used in the analysis will be transcribed and rescaled to a score from 0 to 100 from the raw measurements on the Taste Assessment Questionnaire.

3.4. Baseline Variables

Baseline characteristics will be collected according to the SoA as specified in the protocol.

3.5. Safety Endpoints

The following data will be considered in standard safety summaries (see protocol for collection days, baseline assessment, and list of parameters):

- Adverse events (AE)
- Laboratory data

3.5.1. Adverse Events

An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention.

Any adverse events occurring following start of treatment will be considered as treatment emergent adverse event (TEAE). Events that occur during follow-up within the lag time of up to 37 days after the last dose of study intervention will be counted as treatment emergent. The time period for collecting AEs ("active collection period") for each participant begins from the time the participant provides informed consent.

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 haematological, clinical chemistry (serum) and urinalysis 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 parameter.

The baseline measurement is the predose measurement on Day -1.

4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

Data for all participants will be assessed to determine if participants meet the criteria for inclusion in each analysis population prior to releasing the database and classifications will be documented per standard operating procedures.

Participant Analysis Set	***Description***
Enrolled	*"Enrolled" means a participant's, or their legally authorized representative's, agreement to participate in a clinical study following completion of the informed consent process and assignment to study intervention. A participant will be considered enrolled if the informed consent is not withdrawn prior to participating in any study activity. Potential participants who are screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled, unless otherwise specified by the protocol.*
PK Concentration Analysis Set	*All enrolled participants who receive 1 single IN dose of zavegepant and provide at least 1 evaluable plasma concentration.*
PK Parameter Analysis Set	*All enrolled participants who receive 1 single IN dose of zavegepant and provide at least 1 evaluable PK parameters of interest.*
Taste Assessment Set	*All participants who receive at least 1 single IN dose of zavegepant and complete the Taste Assessment Questionnaire*
Safety Analysis Set	*All participants who take at least 1 dose of study intervention. Participants will be analyzed according to the product they actually receive.*

5. GENERAL METHODOLOGY AND CONVENTIONS

Final analysis will be performed after study participant data set release following last participant last visit.

5.1. Hypotheses and Decision Rules

No statistical hypothesis will be tested in this study.

5.2. General Methods

5.2.1. Analyses for Binary/Categorical Endpoints

For binary or categorical variables, number of participants, numbers and percentages of participants meeting the categorical criteria will be presented in accordance with the Clinical Data Interchange Standards Consortium and Pfizer Standards (CaPS).

5.2.2. Analyses for Continuous Endpoints

For continuous variables, the data will be summarized using the number of participants, mean, median, standard deviation (SD), minimum, and maximum in accordance with the CaPS. For appropriate PK parameters, geometric mean and geometric coefficient of variation (%CV) will also be summarized.

5.3. Methods to Manage Missing Data

5.3.1. Pharmacokinetic Data

Methods to handle missing PK data are described below.

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 and plots of median profiles, statistics will be calculated having set 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 as 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.

An anomalous concentration value is one that, after verification of bioanalytical validity, is grossly inconsistent with other concentration data from the same individual or from other participants. For example, a BLQ concentration that is between quantifiable values from the same dose is considered as anomalous. Anomalous concentration values may be excluded from PK analysis at the discretion of the PK analyst or pharmacokineticist.

PK 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 treatment with ≥3 evaluable measurements. PK parameter analyses will not be performed for a particular parameter if more than 50% of the data are NC.

If an individual participant has a known biased estimate of a PK parameter (due for example to an unexpected event such as vomiting before all the compound is adequately absorbed from the gastrointestinal tract), this will be footnoted in summary tables and will not be included in the calculation of summary statistics or statistical analyses.

5.3.2. Safety Data

For the analysis of safety endpoints, the standard rules for imputation according to CaPS will be applied.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoints

6.1.1. Zavegepant PK Concentrations

The plasma concentrations and plasma equivalent conventrations (derived from Tasso-M20 samples) of zavegepant will be listed and descriptively summarized by nominal PK sampling time, for each collection method (Tasso-Plus, Tasso-M20 and venous phlebotomy), and by race (non-Japanese and Japanese), as data permit for the PK Concentration Analysis Set. Individual participant and summary profiles (mean and median plots) of the plasma concentration-time data will be plotted by collection method and race using actual and nominal times, respectively. Mean and median zavegepant plasma concentration profiles will be presented on both linear and semi-log scales.

Presentations for zavegepant plasma concentrations will include:

- A listing of all concentrations sorted by participant ID, collection method, race and nominal time postdose. The concentration listing will also include the actual times. Deviations from the nominal time will be provided in a separate listing.

- A summary of concentrations by collection method and by race, and nominal time postdose, where the set of statistics will include n, mean, median, SD, %CV, minimum, maximum and the number of concentrations above the LLQ.

- Median concentrations time plots (on both linear and semi-log scales) against nominal time postdose by collection method and by race (all collection methods on the same plot per scale (similarly for race), based on the summary of concentrations by collection method, race and time postdose).

- Mean concentrations time plots (on both linear and semi-log scales) against nominal time postdose by collection method and race (all collection methods on the same plot per scale (similarly for race), based on the summary of concentrations by collection method, race and time postdose).

- Individual concentration time plots by collection method and by race(on both linear and semi-log scales) against actual time postdose (there will be separate spaghetti plots for each collection method and raceper scale).

- Individual concentration time plots by participant (on both linear and semi-log scales) against actual time postdose [there will be separate plots for each participant (containing all collection methods and all race) per scale].

The correlations of paired Tasso-Plus versus venous phlebotomy concentrations and paired Tasso-M20 versus venous phlebotomy concentrations will be reported. Furthermore, Bland-Altman plot analysis will be performed for each pair to evaluate the bias and SD of the bias between the mean differences and to estimate an agreement interval within a 95% confidence limit.

6.1.2. Zavegepant PK Parameters

The PK parameters of zavegepant will be listed and summarized descriptively for each collection method (Tasso-Plus, Tasso-M20 and venous phlebotomy), and by race (non-Japanese and Japanese), as data permits for the PK Parameter Analysis Set. Each zavegepant PK parameter will be summarized including the set of summary statistics as specified in Table 3.

For AUC_{last}, AUC_{inf}, and C_{max}, box and whisker plots for individual participant parameters will be constructed for each collection method and overlaid with geometric means.

Table 3. PK Parameters to be Summarized Descriptively by Collection Method and Race

Parameter	Summary Statistics
AUC_{inf}, AUC_{last}, C_{max}, CL/F, V_z/F	N, arithmetic mean, median, SD, %CV, minimum, maximum, geometric mean and geometric %CV
T_{max}, T_{last}	N, median, minimum, maximum
$t_{½}$	N, arithmetic mean, median, SD, %CV, minimum, maximum

Supporting data from the estimation of $t_{½}$ and AUC_{inf} will be listed by collection method and by race: terminal phase rate constant (k_{el}); goodness of fit statistic from the log-linear regression (r^2); the percent of AUC_{inf} based on extrapolation (AUC_{extrap}%); and the first, last, and number of time points used in the estimation of k_{el}. This data may be included in the clinical study report.

For the Tasso vs. Venous comparison, natural log-transformed zavegepant AUC_{inf} (if data permit), AUC_{last} and C_{max} will be analyzed using a mixed effect model with blood collection method as fixed effect and participant as random effect. Estimates of the adjusted mean differences (Test – Reference) and corresponding 90% CIs will be obtained from the model with Venous as the Reference group and Tasso-M20 (or Tasso-Plus) as the Test group. The adjusted mean differences and 90% CIs for the differences will be exponentiated to estimate the adjusted geometric means ratio (Test/Reference) and 90% CIs for the ratios.

For race comparison, natural log-transformed zavegepant AUC_{inf} (if data permit), AUC_{last} and C_{max} will be analyzed using an ANOVA model with race as fixed effect. Estimates of the adjusted mean differences (Test – Reference) and corresponding 90% CIs will be obtained from the model with non-Japanese as the Reference group and Japanese as the Test group. The adjusted mean differences and 90% CIs for the differences will be exponentiated to estimate the adjusted geometric means ratio (Test/Reference) and 90% CIs for the ratios.

6.2. Secondary Endpoints

6.2.1. Safety Endpoints

Safety data will be analyzed on Safety Analysis Set in accordance with the CaPS (see Section 6.5).

6.2.2. Other Endpoint

The sensory attributes (overall liking, bitterness, tongue/mouth burn, throat burn, sour taste, salty taste, and sweet taste) from the Taste Assessment Questionnaire will be listed and descriptively summarized by study intervention (zavegepant alone vs zavegepant + butterscotch candy) and time points.

Summary statistics (mean and 90% CI) will be calculated for the various questions. Radar plots for each of 4 time points (1, 5, 10 and 20 minutes after dosing), summarizing all attributes for each intervention will be generated. Boxplots of each attribute will be plotted against the time points.

6.3. Subset Analyses

There are no planned subset analyses.

6.4. Baseline and Other Summaries and Analyses

6.4.1. Demographic Summaries

Demographic characteristics will be summarized for enrolled population in accordance with the CaPS.

6.4.2. Study Conduct and Participant Disposition

Participants evaluation groups will show end of study participant disposition. Frequency counts will be supplied for participant discontinuation(s). Data will be reported in accordance with the CaPS.

6.4.3. Study Treatment Exposure

Study treatment exposure will be listed.

6.4.4. Concomitant Medications and Nondrug Treatments

All concomitant medication(s) as well as non-drug treatment(s) will be reported in the listings.

6.5. Safety Summaries and Analyses

All safety analyses will be performed on the Safety Analysis Set.

Safety data will be presented in tabular and/or graphical format and summarized descriptivelyby Tasso device used and by race, where appropriate.

6.5.1. Adverse Events

Adverse events will be reported in accordance with the CaPS and listed by Tasso device used and by race, where appropriate.

Participant discontinuations due to adverse events will be detailed by Tasso device used and by race. Data will be reported in accordance with the CaPS.

6.5.2. Laboratory Data

Laboratory data will be listed and summarized by Tasso device used and by race in accordance with the CaPS.

7. INTERIM ANALYSES

No 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, and/or supporting clinical development.

7.1. Introduction

Not applicable.

7.2. Interim Analyses and Summaries

Available safety and PK data may be reviewed.

APPENDICES

Appendix 1. Summary of Analyses

Endpoint	Population	Data Inclusion and Rules for Handling Intercurrent Events and Missing Data	Analysis Method
Zavegepant PK concentrations	PK Concentration Analysis Set	Observed and imputed (Section 5.3.1) data	Descriptive statistics
Zavegepant PK parameters	PK Parameter Analysis Set	Observed and imputed (Section 5.3.1) data	Descriptive statistics
Safety data	Safety Analysis Set	Observed and imputed (Section 5.3.2) data	Descriptive statistics
Taste assessment data	Taste Assessment Set	Observed data	Descriptive statistics

Appendix 2. List of Abbreviations

Abbreviation	Term
%CV	coefficient of variation
AE	adverse event
AUC_{extrap}%	the percent of AUC_{inf} based on extrapolation
AUC_{inf}	area under the plasma concentration-time profile from time zero extrapolated to infinite time
AUC_{last}	area under the plasma concentration-time profile from time zero to the time of the last quantifiable concentration
BLQ	below the limit of quantitation
B/P	blood to plasma
CaPS	Clinical Data Interchange Standards Consortium and Pfizer Standards
CGRP	calcitonin gene-related peptide
CI	confidence interval
CL/F	apparent clearance
C_{last}	predicted plasma concentration at the last quantifiable time point from the log-linear regression analysis
C_{max}	maximum plasma concentration
GMR	geometric mean ratio
FDA	Food and Drug Administration
IN	intranasal
k_{el}	elimination rate constant estimated from the log-linear regression analysis
LLQ	lower limit of quantitation
N/A	not applicable
NC	not calculated
ND	not done
NS	no sample
PCS	patient-centric sampling
PK	pharmacokinetic(s)
r^2	goodness of fit statistic from the log-linear regression
SAP	statistical analysis plan
SD	standard deviation
SoA	schedule of activities
$t_{1/2}$	terminal plasma elimination half-life
TEAE	treatment emergent adverse event
T_{max}	time for C_{max}
V_z/F	apparent volume of distribution after oral dose