Protocol C5041050

A Phase 1, Open-Label, Single Dose, Fixed-Sequence Crossover Sub Study to Determine the Pharmacokinetics Using Tasso Device and Safety and Tolerability Using Wearable Monitoring Devices Following Single Oral Doses of Etrasimod 2 mg IR Tablets in Healthy Adult Participants in a Hybrid Decentralized Clinical Trial Design

[redacted]

Statistical Analysis Plan
(SAP)

Version: 1

Date: 22 Dec 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 / 22 Dec 2023	Amendment 2 06 Nov 2023	N/A	N/A

2. INTRODUCTION

Etrasimod is an orally administered, selective, synthetic $S1P_{1,4,5}$ modulator that is being developed to treat immune-mediated inflammatory disorders, including UC, AA, AD, CD and EoE. The $S1P_1$ is a cell surface expressed protein that has been shown to regulate lymphocyte migration out of lymphoid tissues. Synthetic small molecule $S1P_1$ agonists have been observed to act as functional antagonists by inducing sustained receptor internalization, thus inhibiting lymphocyte migration out of lymphoid tissues and lowering the amount of peripheral blood lymphocytes available to be recruited to sites of inflammation. Modulation of the S1P/S1P receptor axis is thought to be a potential therapeutic approach to the management of immune-mediated inflammatory disorders.

This sub study is being conducted to assess the feasibility of conducting a Phase 1 etrasimod study as a hybrid decentralized clinical trial with dosing by study personnel at the CRU and remote collection of PK, safety and tolerability data. The specific objectives of the sub study are to determine the PK and to assess the safety and tolerability of etrasimod clinical IR tablets 2 mg under fasted conditions in healthy adult participants in a hybrid DCT design.

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in Study C5041050.

2.1. Modifications to the Analysis Plan Described in the Protocol

PK concentration and parameters pertaining to Tasso OnePlus micro sampling will be regarded as results from serum etrasimod in summary tables and listings, while PK concentration and parameters derived from venous sampling will be regarded as results from plasma etrasimod.

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:***
• *To compare the PK of etrasimod 2 mg clinical IR tablets under fasted conditions in healthy participants using Tasso OnePlus PK micro samples taken by participants only (Treatment G) vs. CRU staff and participants (Treatment F) in a hybrid DCT design*	• *Plasma AUC_{24hr}, $AUC_{24hr\text{-}last}$, AUC_{inf} (if data permit, otherwise AUC_{last}) and C_{max} of etrasimod*
Secondary:	***Secondary:***
• *To compare the PK of etrasimod 2 mg clinical IR tablets under fasted conditions in healthy participants using Tasso OnePlus PK micro samples taken by participants only (Treatment G) vs. CRU staff and participants (Treatment F) in a hybrid DCT design vs. venous PK samples taken by CRU staff in conventional design of main study (Treatment A)*	• *Plasma $AUC_{24\ hr}$, $AUC_{24hr\text{-}last}$, AUC_{inf} (if data permit, otherwise AUC_{last}) and C_{max} of etrasimod*
• *To evaluate the safety and tolerability of etrasimod 2 mg clinical IR tablets in healthy participants using mobile wearable devices in a hybrid DCT design*	• *Assessment of first dose HR reduction, TEAEs, clinical laboratory abnormalities, vital signs, PEs, and ECGs*
Tertiary/Exploratory:	***Tertiary/Exploratory:***
• *To compare the concentrations of etrasimod in PK samples collected using Tasso OnePlus micro sampling device against those using venous blood sampling.*	• *Plasma concentrations of etrasimod obtained via micro sampling compared to venous sampling at pre-dose and at 2 and 4 hrs post-dose.*

2.3. Study Design

This is a Phase 1, open-label, single-dose, fixed-sequence, crossover study in a single cohort of approximately 8 healthy male or female participants conducted using a hybrid DCT design.

The study will consist of 2 treatments. Participants will receive the two Treatments F and G in a fixed sequence as shown below. There will be an 8-day washout between each dose.

Fixed Treatment Sequence (n=8)	***Period 1***	***Period 2***
1	*F*	*G*

Treatment F: Single oral dose of etrasimod 2 mg, DCT with practice sessions (Reference)*
*Treatment G: Single oral dose of etrasimod 2 mg, DCT without practice sessions** (Test)*
** Practice of Tasso and Wearable Monitoring Devices on Day 2 at CRU; participant self-assessment remotely for vitals and ECG (Days 3-5) and PK samples (Days 3-8)*
*** Participant self-assessment using Tasso and Wearable Monitoring Devices on Days 1-2 at CRU; and remotely on Days 3-8*

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoints

The primary endpoints are the plasma AUC_{24hr}, $AUC_{24hr\text{-}last}$, AUC_{inf} (if data permit, otherwise AUC_{last}) and C_{max} of etrasimod. The test/reference ratios for $AUC_{24\ hr}$, $AUC_{24hr\text{-}last}$, AUC_{inf}, AUC_{last} and C_{max} will be derived with Treatment G (etrasimod 2 mg, DCT Tasso PK micro samples taken by participants only) as the test treatment and Treatment F (etrasimod 2 mg, DCT Tasso PK micro samples taken by CRU staff and participants) as the reference treatment.

Plasma PK parameters of etrasimod will be derived (as data permit) from the concentration-time data using standard noncompartmental methods as outlined in the Table 2 below. 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***
AUC_{inf}	*Area under the concentration-time curve from time zero extrapolated to infinity*	*$AUC_{last} + (C_{last}*/k_{el})$, where $C_{last}*$ is the predicted plasma concentration at the last quantifiable time point from the log-linear regression analysis*
AUC_{last}	*Area under the plasma concentration-time profile from time zero to the time of the last quantifiable concentration (C_{last})*	*Linear/Log trapezoidal method.*
AUC_{24hr}	*Area under the plasma concentration-time profile from time zero to 24 hr*	*Linear/Log trapezoidal method.*
$AUC_{24hr\text{-}last}$	*Area under the plasma concentration-time profile from time 24 hr to the time of the last quantifiable concentration (C_{last})*	*Linear/Log trapezoidal method.*
C_{max}	*Maximum observed concentration*	*Observed directly from data*

3.2. Secondary Endpoints

3.2.1. PK Parameters from Tasso and Venous Blood Sampling

The test/reference ratios for AUC_{24hr}, $AUC_{24hr\text{-}last}$, AUC_{inf}, AUC_{last} and C_{max} will be derived with Treatment A (etrasimod 2 mg, Main study, venous PK samples) as the Reference treatment while Treatment F (etrasimod 2 mg, DCT Tasso PK micro samples taken by CRU staff and participants) and Treatment G (etrasimod 2 mg, DCT Tasso PK micro samples taken by participants only) as the Test treatments.

3.2.2. Safety Endpoints

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

- first dose HR reduction
- adverse events (AE)

- laboratory data
- vital signs data
- electrocardiogram (ECG) results

3.2.2.1. First Dose HR reduction

For all periods, HR will be measured every hour up to 6 hours, at 8 and 24 hours post-etrasimod dose at Day 1. The baseline HR value is the average of the HR values from the triplicate ECG measurements collected predose on Day 1 of each period. Changes from baseline will be defined as the change between the postdose measurements and baseline value. In addition, the nadir HR value on Day 1 will be derived.

3.2.2.2. Adverse Events

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 35 days after the last dose of study intervention will be counted as treatment emergent and attributed to the last treatment taken. The time period for collecting AEs ("active collection period") for each participant begins from the time the participant provides informed consent.

3.2.2.3. Laboratory Data

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

For all periods, the baseline measurement is the predose measurement on Day -1 in each period. Changes from baseline will be defined as the change between the postdose and baseline measurements.

3.2.2.4. Vital Signs

Supine blood pressure (BP), pulse rate (PR) and temperature will be measured at times specified in the SoA given in the protocol.

For all periods, the baseline measurement is the predose measurement on Day 1 in each period. Changes from baseline will be defined as the change between the postdose and baseline measurements.

3.2.2.5. Electrocardiograms

QT interval, QTcF, PR interval, QRS and heart rate (HR) will be recorded at each 12-lead ECG assessment time indicated in the SoA given in the protocol. QTcF will be derived using Fridericia's heart rate correction formula:

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

For all periods, the baseline value is the average of triplicate ECG measurements collected predose on Day 1 of each period. Changes from baseline will be defined as the change between the postdose measurements and baseline value.

3.3. Other Safety Endpoint(s)

None.

3.4. Exploratory Endpoint

Plasma concentrations of etrasimod obtained via micro sampling (taken by participants only and taken by CRU staff and participants) compared to venous sampling at predose and at 2 and 4 hours postdose.

3.5. Baseline Variables

Baseline characteristics will be collected according to the schedule of activities (SoA) as specified in the protocol.

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***
Safety Analysis Set	*All participants who take at least 1 dose of study intervention. Participants will be analyzed according to the product they actually received.*
PK Concentration Set	*All participants who take at least 1 dose of study intervention and in whom at least 1 concentration value is reported.*
PK Parameter Set	*All participants who take at least 1 dose of study intervention and in whom at least 1 of the PK parameters of interest are reported.*

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 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.

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 not be presented for a particular treatment group if more than 50% of the data are NC. For statistical analyses, PK parameters coded as NC will also be set to missing, and statistics will be presented for a particular treatment with $\geq$3 evaluable measurements.

If an individual participant has a known biased estimate of a PK parameter (due for example to a dosing error or 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. For instance, if a participant has a vomiting event post dose that is within a duration of time that is 2-times the derived median T_{max} for the population for the administered treatment, then the pharmacokineticist should consider the exclusion of the PK concentration data collected following the initial vomiting event in that treatment period and the PK parameter data reported for that treatment period from the datasets used to calculate summary statistics or statistical analyses.

5.3.2. Safety Data

Missing values in standard summaries of safety data will be imputed according to CaPS.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoints

Plasma AUC_{24hr}, $AUC_{24hr\text{-}last}$, AUC_{last}, AUC_{inf} and C_{max} of etrasimod will be summarized by treatment group and will include the set of summary statistics as specified in Table 3.

Table 3. PK Parameters to be Summarized Descriptively by Treatment

Parameter	Summary Statistics
AUC_{24hr}, $AUC_{24hr\text{-}last}$, AUC_{inf}, AUC_{last}, C_{max}	N, arithmetic mean, median, SD, %CV, minimum, maximum, geometric mean and geometric %CV

Natural log transformed AUC_{24hr}, $AUC_{24hr\text{-}last}$, AUC_{inf}, AUC_{last}, and C_{max} will be analyzed using a mixed effect model with treatment as a 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. The adjusted mean differences and 90% CIs for the differences will be exponentiated to provide estimates of the ratio of adjusted geometric means (Test/Reference) and 90% CI for the ratios. Treatment F (etrasimod 2 mg, DCT Tasso PK micro samples taken by CRU staff and participants) will be the Reference treatment while Treatment G (etrasimod 2 mg, DCT Tasso PK micro samples taken by participants only) will be the Test treatment.

A listing of the individual participant ratios (Test/Reference) will be provided. Box and whisker plots for AUC_{24hr}, $AUC_{24hr\text{-}last}$, AUC_{inf}, AUC_{last} and C_{max}, will be plotted by treatment and overlaid with geometric means.

Residuals from the models will be examined for normality and the presence of outliers via visual inspection of plots of residuals vs predicted values and normal probability plots of residuals but these will not be included in the CSR. If there are major deviations from normality or outliers then the effect of these on the conclusions will be investigated through alternative transformations and/or analyses excluding outliers. Justification for any alternative to the planned analysis will be given in the report of the study.

6.2. Secondary Endpoints

6.2.1. PK Parameters from Tasso and Venous Blood Sampling

Natural log transformed AUC_{24hr}, $AUC_{24hr\text{-}last}$, AUC_{inf}, AUC_{last}, and C_{max} will be analyzed using an ANOVA with treatment as a fixed effect. Estimates of the adjusted mean differences (Test-Reference) and corresponding 90% CIs will be obtained from the model. The adjusted mean differences and 90% CIs for the differences will be exponentiated to provide estimates of the ratio of adjusted geometric means (Test/Reference) and 90% CI for the ratios. Treatment A (etrasimod 2 mg, Main study, venous PK samples) will be the Reference treatment while Treatment F (etrasimod 2 mg, DCT Tasso PK micro samples taken by CRU staff and participants) and Treatment G (etrasimod 2 mg, DCT Tasso PK micro samples taken by participants only) will be the Test treatments.

6.2.2. Safety Endpoints

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

Safety data will be presented in tabular and/or graphical format and summarized descriptively, where appropriate.

6.2.2.1. First Dose HR reduction

Changes from predose baseline in HR at every hour up to 6 hours, at 8 and 24 hours on Day 1 and on Days 3 to 5, as well as the nadir HR value on Day 1 of each period, will be summarized by treatment.

6.2.2.2. Adverse Events

TEAEs will be reported in accordance with the CaPS.

Participant discontinuations due to adverse events will be detailed by treatment. Data will be reported in accordance with the CaPS.

6.2.2.3. Laboratory Data

Laboratory data will be listed and summarized by treatment in accordance with the CaPS.

6.2.2.4. Vital Signs

Vital sign data will be listed and summarized by treatment in accordance with the CaPS.

6.2.2.5. Electrocardiograms

ECG data will be listed and summarized by treatment in accordance with the CaPS.

Changes from baseline for the ECG parameters will be summarized by treatment. The number (%) of participants with maximum postdose QTcF values and maximum increases from baseline in the following categories will be tabulated by treatment.

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

6.3. Other Safety Summaries and Analyses Endpoint(s)

None.

6.4. Exploratory Endpoint

6.4.1. Plasma Concentrations of Etrasimod

The plasma concentrations will be listed and descriptively summarized by nominal PK sampling time and treatment. Individual participant, as well as mean and median profiles of the plasma concentration-time data will be plotted by treatment using actual (for individual) and nominal (for mean and median) times respectively. Mean and median profiles will be presented on both linear and semi-log scales.

Presentations of concentrations will include:

- A listing of all concentrations sorted by participant 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.

- A summary of concentrations by treatment 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 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 postdose 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).

- 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 treatments) per scale].

Etrasimod concentrations at the predose and 2- and 4-hour postdose sampling time points from paired samples obtained via micro sampling and venous sampling will be compared. Individual participant differences and % differences of concentrations by PK sampling time, will be listed and summarized descriptively.

6.5. Subset Analyses

There are no planned subset analyses.

6.6. Baseline and Other Summaries and Analyses

6.6.1. Demographic Summaries

Demographic characteristics will be summarized for Safety Analysis Set in accordance with the CaPS.

6.6.2. Study Conduct and Participant Disposition

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

6.6.3. Study Treatment Exposure

Study treatment exposure will be listed.

6.6.4. Concomitant Medications and Nondrug Treatments

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

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, facilitating PK/PD modeling, and/or supporting clinical development.

Final analysis will follow the official database release. As this will be an open-label study, there is no formal unblinding of the randomization code.

Appendix 1. SAS Code for Analyses

An example of the PROC MIXED code is provided below:

For the primary objective (G vs F):

```
proc mixed data=tab.pk;
     class trt participant;
     model l&var= trt/ ddfm=KR;
     random participant/subject=participant;
     lsmeans trt;
     estimate 'G vs F' trt -1 1  /cl alpha=0.1;

     ods 'Estimates' out=est&var;
     ods 'lsmeans' out=ls&var;
     ods 'covparms' out=cov&var;
     ods 'tests3' out=tst&var;
run;
```

For the secondary objective (G or F vs A):

```
proc mixed data=tab.pk;
     class trt;
     model l&var= trt/ ddfm=KR;
     lsmeans trt;
     estimate 'F vs A' trt -1 1 0 /cl alpha=0.1;
     estimate 'G vs A' trt -1 0 1  /cl alpha=0.1;

     ods 'Estimates' out=est&var;
     ods 'lsmeans' out=ls&var;
     ods 'covparms' out=cov&var;
     ods 'tests3' out=tst&var;
run;
```

/* Letter assignments for treatments (trt) within the estimate statement above are as follows
Treatment A: Single oral dose of etrasimod 2 mg clinical IR tablet under fasted conditions (from C5041034, Reference for the secondary endpoint analysis)

Treatment F: Single oral dose of etrasimod 2 mg, DCT with practice sessions [DCT Tasso PK micro samples taken by CRU staff and participants] (Reference for the primary endpoint analysis, Test for the secondary endpoint analysis)

Treatment G: Single oral dose of etrasimod 2 mg, DCT without practice sessions [DCT Tasso PK micro samples taken by participants only] (Test for the primary and secondary endpoint analyses) */

Appendix 2. List of Abbreviations

Abbreviation	Term
%CV	coefficient of variation
AA	alopecia areata
AD	atopic dermatitis
AE	adverse event
ANOVA	analysis of variance
AUC_{24hr}	area under the plasma concentration-time profile from time zero to 24 hr
$AUC_{24hr\text{-}last}$	Area under the plasma concentration-time profile from time 24 hr to the time of the last quantifiable concentration
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 quantification
BP	blood pressure
CaPS	Clinical Data Interchange Standards Consortium and Pfizer Standards
CD	Crohn's Disease
CI	confidence interval
C_{last}	last quantifiable concentration
C_{max}	maximum plasma concentration
CRU	clinical research unit
CSR	clinical study report
DCT	decentralized clinical trial
ECG	electrocardiogram
EoE	eosinophilic esophagitis
HR	heart rate
IR	immediate-release
k_{el}	terminal phase rate constant
LLQ	lower limit of quantification
mg	milligram
ms	millisecond
N/A	not applicable
NC	not calculated
ND	not done
NS	no sample
PD	pharmacodynamic(s)
PE	physical examination
PK	pharmacokinetic(s)
PR	pulse rate
PR interval	time from the beginning of the P wave to the beginning of the QRS complex
QRS	combination of Q-, R- and S- wave on an electrocardiogram representing ventricular depolarization
QT	time from the start of the Q- wave to the end of T- wave, which represents time taken for ventricular depolarization and repolarization
QTc	corrected QT
QTcF	corrected QT (Fridericia method)
RR	time between the start of one QRS complex and the start of the next QRS complex
SAP	statistical analysis plan
SD	standard deviation
S1P	sphingosine 1-phosphate
$S1P_{1,4,5}$	S1P receptors 1, 4, and 5

Abbreviation	Term
SoA	schedule of activities
TEAE	treatment emergent adverse event
T_{max}	time to C_{max}
UC	ulcerative colitis