Protocol C5241015

A PHASE 1, RANDOMIZED, DOUBLE-BLIND, SPONSOR-OPEN, PLACEBO- AND POSITIVE-CONTROLLED CROSSOVER STUDY TO INVESTIGATE THE EFFECT OF MULTIPLE DOSES OF SISUNATOVIR ON QTC INTERVAL IN HEALTHY ADULT PARTICIPANTS

Statistical Analysis Plan
(SAP)

Version: **1**

Date: 05 Jun 2023

Note: Text taken verbatim from the protocol is *italicized* in this document.

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1. VERSION HISTORY

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
5 Jun 2023	Amendment 1 01 May 2023	N/A	N/A

2. INTRODUCTION

This study is designed as a thorough QT study (TQT) to assess the effect of sisunatovir on time from the beginning of the QRS complex to the end of the T wave on the electrocardiogram (ECG), corresponding to electrical systole (QT interval) as recommended by the International Council for Harmonisation (ICH) E14 guideline and in Q&As (R3).

This statistical analysis plan (SAP) provides the detailed methodology for summary and statistical analyses of the data collected in Study C5241015. 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. Modifications to the Analysis Plan Described in the Protocol

Not Applicable.

2.2. Study Objectives, Endpoints, and Estimands

There are no estimands for this study.

Type	Objective	Endpoint
Primary	*Primary*	*Primary*
ECG (QT)	• *To evaluate the effect on QT interval corrected for heart rate (QTc) interval in healthy participants, of possible clinical therapeutic concentrations of sisunatovir compared with placebo.*	• *Placebo-corrected, baseline adjusted QTc interval using Fridericia's correction method (QTcF) of sisunatovir at expected maximum concentrations (Cmax) at the* [redacted].
Secondary	*Secondary*	*Secondary*
Safety (Section 6.6)	• *To evaluate the safety and tolerability at possible clinical therapeutic concentrations of sisunatovir, including but not limited to cardiovascular safety.*	• *Adverse event (AE), ECG assessments, vital sign measurements, and clinical safety laboratory measurements.*

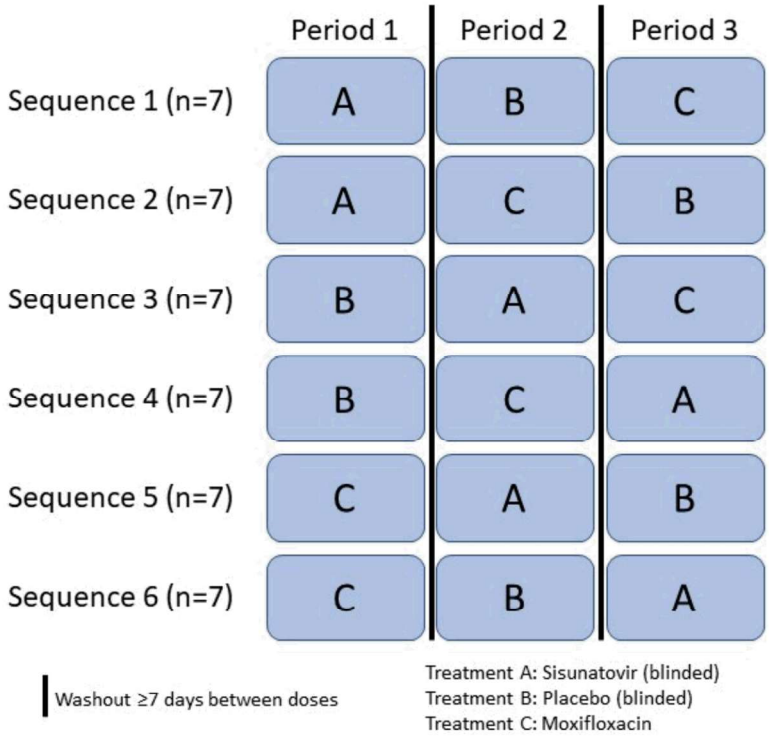
ECG (QT)	• *To determine study sensitivity by comparing the effect of moxifloxacin 400 mg on QTc interval with placebo at historical moxifloxacin time for maximum observed concentration (T_{max}) of 3-5 hours.*	• *Baseline adjusted QTcF of moxifloxacin and placebo at T_{max} of 3, 4, and 5 hours on Day 3.*
Exploratory	*Exploratory*	*Exploratory*
ECG	• *To evaluate the effect of sisunatovir on the electrocardiogram (ECG) parameters of, heart rate, PR interval and QRS interval.*	• *Baseline adjusted heart rate, PR interval, and QRS intervals of sisunatovir and placebo at each postdose time point.*
Pharmacokinetics (PK)	• *To evaluate the plasma PK of sisunatovir following multiple oral doses.*	• *Plasma sisunatovir parameters, including C_{max}, T_{max} and area under the plasma concentration-time profile from time 0 to 24 hours (AUC_{24}) post-dose on Day 3.*
PK	• *To explore PK of sisunatovir by microsampling technique.*	• *Concentration of sisunatovir from paired samples obtained via microsampling compared to venous sampling.*

2.3. Study Design

This is a Phase 1, multiple-dose, randomized, 3-treatment, 3-period cross-over, 6-sequence, sponsor-open, placebo-and positive-controlled study to be conducted in approximately 42 adult healthy participants under fed conditions. Approximately 42 participants will be randomized into 6 sequences with 7 participants in each sequence. Participants assigned to treatment A will receive [redacted] oral doses of sisunatovir administered Q12 hours over [redacted] days in a fed state. Participants assigned to treatment B will receive [redacted] oral doses of matching placebo administered Q12 hours over [redacted] days in a fed state. Participants assigned to treatment C will receive [redacted] oral doses of placebo administered Q12 hours for [redacted] days followed by a single dose of 400 mg moxifloxacin on the morning of Day [redacted]. Treatment assignments to sisunatovir and placebo will be blinded to the participants, investigator and CRU staff (except pharmacy staff) but open to the sponsor. Moxifloxacin administration on Day [redacted] will be unblinded.

The study schema is shown below in Figure 1.

Figure 1. Study Schema



3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoint(s)

3.1.1. Placebo-corrected, baseline adjusted QTc interval using Fridericia's correction method (QTcF) of sisunatovir at expected maximum concentrations (C_{max}) at the CCI [redacted].

The average of the triplicate ECGs collected at each time point specified in the protocol *will be calculated and used in PK/PD modeling, statistical analyses, and/or descriptive summaries.* Missing ECG measurements will not be imputed.

The baseline of ECG parameters will be defined as the mean of the 3 averages of the triplicate measurements taken at the following 3 time points (approximately 40 min, 25 min, and 10 min before pre-dose meal) before dosing on Day 1 within each period. Missing ECG measurements will not be imputed.

The QTcF is defined as Fridericia's heart rate correction, obtained using the formula:

- *QTcF (msec) = QT (msec)/[RR*(sec)$]^{1/3}$ *where RR(sec) = 60/HR (if not provided)*

Change from baseline QTcF will be calculated for each participant at each post-dose timepoint in each period. The placebo-corrected change from baseline QTcF of sisunatovir at

expected Cmax at the CCI [redacted] will be obtained from the concentration-QT (c-QT) analysis as described in Section 5.2.3.

3.2. Secondary Endpoint(s)

3.2.1. Safety Endpoints

3.2.1.1. Adverse Events

An adverse event is considered a Treatment-Emergent Adverse Event (TEAE) if the event started during the effective duration of treatment. All events that start on or after the first dosing day and time/start time, if collected, but before the end of the study will be flagged as TEAEs. The algorithm will not consider any events that started prior to the first dose date.

3.2.1.2. ECG Assessments

A single 12-lead ECG will be obtained on all participants at screening. Triplicate 12-lead ECGs will be collected at all other times as specified in the protocol.

Changes from baseline ECG parameters, including changes from baseline in QTcF, uncorrected QT, QTcB, PR, QRS, and heart rate, will be calculated for each participant and treatment at each postdose timepoint on Day 3 and 4 within each period. See Section 3.1.1 for baseline definition.

The QTcB is the Bazett's heart rate correction, obtained using the formula:

- *$QTcB(msec) = QT(msec)/(RR)^{½}$ where RR(sec) = 60/HR (if not provided)*

The maximum absolute post-dose value and the maximum increase from baseline for QTcF, QTcB, PR, and QRS intervals will be determined for each participant and treatment over the respective period*. When there is no increase from baseline over the respective period, the minimum decrease will be taken.*

3.2.1.3. Vital Sign Measurements

A single measurement of pulse rate and supine systolic and diastolic blood pressure (BP) will be taken at the times specified in the protocol.

Baseline will be the last pre-dose measurement in each study period. Change from baseline will be calculated for all post-baseline timepoints.

3.2.1.4. Laboratory Measurements

Safety laboratory tests (hematology, chemistry, and other clinical laboratory tests) will be performed as described in the protocol. Baseline will be the last pre-dose measurement in each study period. Change from baseline will be calculated for all post-baseline timepoints.

To determine if there are any clinically significant laboratory abnormalities, the safety laboratory tests will be assessed against the criteria specified in the sponsor reporting standards. The assessment will take into account whether each participant's baseline test result is within or outside the laboratory reference range for the particular laboratory parameter.

3.2.2. Baseline adjusted QTcF of moxifloxacin and placebo at T_{max} of 3, 4, and 5 hours on Day 3.

Change from baseline QTcF for each participant at 3, 4, and 5-hours post dosing on Day 3 in the treatment periods of moxifloxacin and placebo will be calculated. See Section 3.1.1 for baseline and calculation details.

3.3. Other Endpoint(s)

3.3.1. Baseline adjusted heart rate, PR interval, and QRS intervals of sisunatovir and placebo at each postdose time point.

Changes from baseline in heart rate, PR interval, and QRS interval of each participants in the treatment periods of sisunatovir and placebo at each postdose time point will be calculated. See Section 3.1.1 for baseline and calculation details.

3.3.2. Plasma sisunatovir parameters, including C_{max}, T_{max} and area under the plasma concentration-time profile from time 0 to 24 hours (AUC_{24}) post-dose on Day 3.

Blood samples for the PK analysis of sisunatovir will be collected following the 0 hour and post-dose ECG measurements at the same time-points on Day 3 for each treatment as specified in the protocol.

The PK parameters of sisunatovir to be derived (if data permit) from the concentration-time data using standard noncompartmental methods are defined 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. Noncompartmental PK Parameters

Parameter	Analysis Scale	Sisunatovir
AUC_{24}	ln	D
C_{max}	ln	D
T_{max}	R	D

Key: ln=natural-log transformed, R=raw (untransformed),
D=displayed with descriptive statistics, A=analyzed using statistical model

3.3.3. Concentration of sisunatovir from paired samples obtained via microsampling compared to venous sampling.

Day 2 PK samples (traditional venous collection) and time matched PK microsamples (dried blood using the Tasso® M20 device) will be collected in Period 1 only at time points specified in the SoA of the protocol. Furthermore, at the 5 hour and 12-hour time point on Day 2, an aliquot of the PK blood sample collection will be taken and used to determine the concentration of sisunatovir in whole blood.

For each individual, blood to plasma (B/P) ratios will be calculated by dividing concurrent (i.e. Day 2, at 5 hour and 12 hour post-dose) whole blood sisunatovir concentration with the plasma sisunatovir concentration and the geometric mean B/P ratio will be calculated. A

population geometric mean of the B/P ratio from all individuals will also be calculated. Missing values will not be imputed. The plasma equivalent sisunatovir concentration by microsampling will be calculated by dividing the whole blood sisunatovir concentration with the individual geometric mean B/P ratio. As a sensitivity analysis, the plasma equivalent concentration will also be calculated using the population geometric mean B/P ratio. AUC_{12} and C_{max} will be derived from blood samples collected using the Tasso® M20 device, and then adjusted for B/P ratio.

3.4. Baseline Variables

- Baseline QTcF covariate for c-QT analysis

Baseline will be defined as the mean of the 3 average triplicate measurements taken at approximately 40 min, 25 min, and 10 min before the pre-dose meal on Day 1 within each period. Denote the baseline QTcF of *participant i in treatment j at time* 0 as $QTcF_{ij0}$. The centered baseline, $QTc_{ij0} - \overline{QTc_0}$ will be used in the c-QT analysis, where $\overline{\mathrm{QTc}_0}$ is the *overall mean of* $QTcF_{ij0}$*, i.e., the mean of all the baseline (= time 0) QTcF values* from all participants. More details will be documented in a separate report and included as an Appendix to the CSR.

- Baseline ECG covariate for MMRM analysis

The pre-dose baselines (one for each treatment period) will be included as covariate in the MMRM analysis of ECG parameters.

3.5. Safety Endpoints

See Section 3.2.1.

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 unblinding and releasing the database and classifications will be documented per standard operating procedures.

Population	***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 randomization. A participant will be considered enrolled if the informed consent is not withdrawn prior to participating in any study activity after screening. 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.*

Population	Description
ECG analysis set	*All participants randomized and treated who have at least 1 post-dose ECG measurement in at least 1 period. Analysis sets may contain different numbers of participants for different ECG parameters based on availability of data. Participants will be analyzed according to the product they actually received.*
Concentration-QTc analysis set	*The concentration QTc analysis population is defined as all participants randomized and treated with sisunatovir or placebo who have at least 1 pair of time-matched post-dose QT and sisunatovir plasma concentration values in at least 1 period of the study. For placebo treatment, the concentration of is set to 0.*
PK Concentration analysis set	*All participants randomly assigned to study intervention and who take at least 1 dose of study intervention and in whom at least 1 concentration value can be reported.*
PK Parameter analysis set	*All participants randomly assigned to study intervention and who take at least 1 dose of study intervention and in whom at least 1 of the PK parameters of interest can be reported.*
Safety analysis set	*All participants randomly assigned to study intervention and who take at least 1 dose of study intervention. Participants will be analyzed according to the product they actually received.*

5. GENERAL METHODOLOGY AND CONVENTIONS

5.1. Hypotheses and Decision Rules

5.1.1. Hypotheses and Decision Rules of the Primary Endpoint

Concentration-QTc analysis will be used to assess if sisunatovir at maximum concentrations (Cmax) at the CCI [redacted] *has clinically relevant effect on the QTc interval at one sided $\alpha = 0.05$ level without multiplicity adjustment. After a final concentration-ΔQTc model has been established, denote the population mean of model predicted placebo-corrected ΔQTcF (ΔΔQTcF) at the Cmax of a dose of interest as μ_{cmax}, the null hypothesis of sisunatovir having a positive QTc effect and the alternative hypothesis of sisunatovir having a negative QTc effect at the dose of interest can be expressed as the following:*

H_0: $\mu_{cmax} \geq 10$ msec

H_a: $\mu_{cmax} < 10$ msec

The lack of effect of sisunatovir on QTc intervals will be concluded if the upper bound of the 2-sided 90% CI for the ΔΔQTcF estimated by the final concentration-ΔQTc model is <10 msec at the highest clinically relevant exposure.

5.1.2. Hypotheses and Decision Rules of Assay Sensitivity

The assay sensitivity is tested at 3, 4 and 5-hours pose-dose of positive control, moxifloxacin,

on Day 3 simultaneously at one-sided α = 0.05 level with Bonferonni correction of α/3 for multiple assessments. Denote the population mean of change from baseline in QTcF of moxifloxacin and placebo at 3, 4, or 5-hr post-dose as μ(M) and μ(P), respectively, the statistical hypotheses are:

H_0: *μ(M) - μ(P) ≤ 5 msec*

H_a: *μ(M) - μ(P) > 5 msec*

The study will be deemed adequately sensitive to detect QT/QTc prolongation if any of the lower limits of the two-sided Bonferroni-adjusted 90% CI (96.7% CI) for mean differences between moxifloxacin and placebo at any of the 3, 4 and 5-hr pose-dose, respectively, are greater than 5 msec.

5.2. General Methods

The analyses related to the primary, secondary and exploratory endpoints will be based on the appropriate population for analysis (see Section 4).

Unless otherwise stated, all summaries and plots will be presented by treatment group. The following treatment group labels (or similar) will be used:

- Placebo
- Sisunatovir
- Moxifloxacin

In this study, an initial dose of CCI *has been selected to achieve high clinical exposure. If observed exposure of* CCI *is lower than predicted and do not cover the high clinical exposure, then the dose will be increased to* CCI. If the dose of CCI is selected, the sisunatovir treatment group will include participants in both CCI mg and CCI mg CCI doses.

5.2.1. Analyses for Continuous Endpoints

Unless otherwise stated, continuous variables will be presented using summary statistics: number of observations, arithmetic mean, standard deviation (SD), median and range (minimum and maximum) values. For endpoints to be analyzed on the natural log scale ($\log_e$), the geometric mean and geometric coefficient of variation (CV) will additionally be calculated.

5.2.2. Analyses for Categorical Endpoints

Categorical variables will be presented using summary statistics: number of observations and percentages.

5.2.3. Concentration-QTc analysis

PK/PD model will be established to characterize the relationship between sisunatovir plasma concentration and change from baseline in Fridericia's heart-rate corrected QT interval

(ΔQTcF) using the concentriaon-QTc analysis set. Based on the recommendation in the White Paper[1], the linear mixed effect model preferred is pre-specified as:

$$\Delta QTcF_{ijk} = \theta_0 + \eta_{0,i} + \theta_1 TRT_j + (\theta_2 + \eta_{1,i})C_{ijk} + \theta_{3k} + \theta_4\left(QTc_{ij0} - \overline{QTc_0}\right) + \varepsilon_{ijk}$$

$\Delta QTcF_{ijk}$ is the change from baseline in QTcF for participant i in treatment j at time k; θ_0 is the population mean intercept in the absence of a treatment effect; $\eta_{0,i}$ is the random effect associated with the intercept term θ_0; θ_1 is the fixed effect associated with treatment TRT_j (0 = placebo, 1 = sisunatovir); θ_2 is the population mean slope of the assumed linear association between plasma sisunatovir concentration and $\Delta QTcF_{ijk}$; $\eta_{1,i}$ is the random effect associated with the slope θ_2; C_{ijk} is the plasma concentration of sisunatovir for participant i in treatment j and time k and C_{ijk} = 0 for placebo group; θ_{3k} is the fixed effect associated with time, where time is a categorical factor*; and θ_4 is the fixed effect associated with centered baseline $QTcF_{ij0}$, where $\overline{QTc_0}$ is overall mean of $QTcF_{ij0}$, i.e., the mean of all the baseline (= time 0) QTcF values. It is assumed the random effects ($\eta_{0,i}$, $\eta_{1,i}$) are normally distributed with mean [0,0] and an unstructured covariance matrix, whereas the residuals ε_{ijk} are normally distributed with mean 0 and variance σ^2.*

The final c-QTc model will be used to compute the placebo adjusted change from baseline QTcF (ΔΔQTcF) and corresponding two-sided 90% CI at concentrations of interest (e.g. *expected C_{max} at the* CCI [redacted] *and potentially high clinical exposures)*. The ΔΔQTcF is the difference between the model-estimated ΔQTcF at concentration of interest and the model-estimated ΔQTcF for placebo (eg. concentration=0). *If the upper bound of the two-sided 90% interval for the ΔΔQTcF as estimated by the final concentration-ΔQTc model established in this study is <10 msec at the highest clinically relevant exposure, the absence of an effect of sisunatovir on QTc will be concluded.*

Evaluation of Assumptions - Linear Mixed Effect Model

Before proceeding to c-QTc modeling, the following four assumptions for the linear mixed effect model will be evaluated using exploratory plots based on the recommendation in the White Paper[1].

- Assumption 1: no drug effect on HR.

  The time course of mean change from baseline in HR and placebo-adjusted HR by treatment will be plotted to evaluate the potential effect of sisunatovir on HR.

- Assumption 2: QTcF interval is independent of HR.

  A scatterplot of QTcF vs. RR intervals by treatment and a QTcF-RR quantile plot will be generated to confirm the appropriateness of Fredericia's heart-rate correction method.

- Assumption 3: No time delay between drug concentration and ΔQTcF.

  PK/PD hysteresis will evaluated using following exploratory plots:

  - Longitidinal plot of mean and 90% CI for ΔQTcF and/or placebo-adjusted ΔQTcF (ΔΔQTcF) and concentration of sisunatovir.

- Plot of mean ΔΔQTc and concentration connected in temporal order for the sisunatovir treatment group.
- Scatter plot of paired ΔΔQTcF and concentration for the sisunatovir treatment group.

- Assumption 4: Linear c-QTcF relationship.

The linearity will be assessed by a scatter plot of paired ΔΔQTcF and concentration by treatment incorporating a trend line (eg, loess smooth or linear regression).

Concentration-QTc Quantile Plot

A concentration-QTc quantile plot of observed data overlaid with the model predictions is a visual assessment of how well the model fits the data. In this plot, the observed drug concentrations are categorized into their deciles (eg, 10 bins of equal size) and the placebo-adjusted mean change from baseline QTcF intervals (ΔΔQTcF) are calculated for each bin, along with their 5th and 95th percentiles, as well as the 90% confidence interval for the mean

Model Selection and Evaluation

If exploratory plots indicate the modeling assumptions for the linear model are not met, additional modeling steps will be performed to determine an appropriate c-QTc model.

- If there is a potential delay between sisunatovir concentration and the effect on QTcF, an alternative model with a delayed effect will be considered to account for the delay.
- If apparent relationship between QTcF and RR interval and/or differences distribution of HR between on- and off-drug conditions are observed in exploratory plots, other approaches to evaluate QT/QTc may be considered as summarized in the methodology paper by Garnett et al[2].
- If a linear relationship between drug concentration and the effect on QTcF is not supported by exploratory plots, or linear models cannot describe the observed data, nonlinear models may be explored to optimize the model fit, including Emax model described below or other types of PD models.

Emax Model

The following Emax model may be fitted and assessed if the linear mixed model clearly does not capture the trend of the data in the concentration-QTcF quantile plot:

$$\Delta QTcF_{ijk} = \theta_0 + \eta_{0,i} + \theta_1 TRT_j + \frac{(\theta_{22} + \eta_{1,i})C_{ijk}}{\theta_{21} + C_{ijk}} + \theta_{3k} + \theta_4\left(QTc_{ij0} - \overline{QTc_0}\right) + \varepsilon_{ijk}$$

The parameter θ_{21} is the population mean of EC_{50}. The parameter θ_{22} is the population mean of maximum effect (Emax) with an associated random effect of $\eta_{1,i}$. The treatment

effect (θ1) may or may not be included in the Emax model. The other parameters are the same as described earlier in the linear mixed effect model.

The goodness-of-fit of the Emax model will be compared with the linear mixed effect model through review of the AIC criteria, the parameter estimates, residual plots, concentration-QTc quantile plots (described below) and other diagnostic plots.

5.2.4. Mixed Models Repeated Measures (MMRM) Analysis

Change from baseline in QTcF intervals will be analyzed using a MMRM model with sequence, period, treatment, time (post-dose timepoint) and treatment by time interaction as fixed effects, participants as a random effect, and baseline QTcF as the covariate. A compound symmetry covariance matrix will be fitted to the repeated times within participant (other covariance matrices will be considered if necessary), and the Kenward-Roger approximation will be used for estimating degrees of freedom. The ECG analysis set will be used for the MMRM analysis.

Standard SAS output will be provided to support the statistical summary table for the analysis model, but will not be included in the CSR.

Example SAS code is provided in Appendix 2.

Statistical Model Diagnostics

The presence of outliers will be investigated for this model. An outlier will be defined as any response data value with a studentized (conditional) residual greater than 3, or less than -3. A listing will be presented of any participants meeting these criteria and will be included with standard SAS output. The assumptions of normality will be verified graphically using residual plots. For each fitted model, a set of conditional studentized residual plots will be produced, including residual plot, histogram of normality, quantile-quantile (Q-Q) plot and summary of fit statistics. The residual plots will not be included in the CSR.

If there are outliers or major deviations from normality, then the effect of these on the conclusions may 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.

5.3. Missing Data

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

In all PK data presentations (except listings), concentrations below the limit of quantification (BLQ) will be set to zero.

In listings, BLQ values (for PK or PD) will be reported as "<LLOQ", where LLOQ will be replaced with the value for the LLOQ.

For PK and PD 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 (i.e. not done) or NS (i.e. no sample),
2. A deviation in sampling time is of sufficient concern or a concentration has been flagged anomalous by the pharmacokineticist/statistician.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoint(s)

6.1.1. *Placebo-corrected, baseline adjusted QTc interval using Fridericia's correction method (QTcF) of sisunatovir at expected maximum oncentrations (Cmax) at the* CCI [redacted].

6.1.1.1. Main Analysis

Change from baseline in QTcF at Day 3 and 4 post-dose will be analyzed by c-QT analysis as described in Section 5.2.3 using the cQTc analysis set. The final c-QTc model parameters will be presented in tabular format showing the estimate, standard error of the estimate, and 95% confidence interval. Goodness-of-fit plots will be presented for the final c-QTc model. A visualization displaying the model predicted mean ΔΔQTcF and the 90% confidence interval will also be created.

Results of the model-based c-QTc analyses will be summarized in the CSR. More details of the model-based analyses, such as exploratory plots, model evaluations, and model fitting statistics, will be documented in a separate report and included as an Appendix to the CSR.

6.1.1.2. Sensitivity/Supplementary Analyses

By time analysis (intersection-union test) will be provided as the supplementary analyses. Briefly, change from baseline in QTcF will be analyzed by MMRM as described in Section 5.2.4 using ECG analysis set. All planned post-dose time points on Day 3 and 4 of each period will be included in the model. The least-squares (LS) means of change from baseline QTcF of sisunatovir and placebo, as well as the ΔΔQTcF, 5.2.1and the corresponding 90% CIs will be reported for each post-dose timepoint. The LS means and 90% CI of ΔΔQTcF (Y-axis) will be plotted against time post dose (X-axis).

6.2. Secondary Endpoint(s)

6.2.1. *Adverse event (AE), ECG assessments, vital sign measurements, and clinical safety laboratory measurements.*

Analysis of AE, ECG assessments, vital sign measurements and clinical safety laboratory measurements will use the Safety Analysis Set defined in Section 4 unless otherwise stated.

6.2.1.1. Adverse event (AE)

Adverse events will be reported in accordance with the sponsor reporting standards.

6.2.1.2. ECG assessments

Absolute values and changes from baseline for the ECG parameters (ie, HR, PR interval, QT interval, QTcF, QTcB, and QRS complex) will be summarized by treatment group and timepoint according to sponsor reporting standards. Tables will be paged by parameter.

Maximum absolute values and changes from baseline for QTcF, QTcB, PR, and QRS, as well as the maximum and minimum absolute values of HR, will also be summarized descriptively by treatment group using categories as defined in Appendix 1. Numbers and percentages of participants meeting the categorical criteria will be provided. All planned and unplanned postdose timepoints will be counted in these categorical summaries.

If any of the 3 individual ECG tracings has a QTcF value ≥ 500 msec, but the mean of the triplicates is < 500 msec, the data from the participant's individual tracing will be described in a safety section of the study report in order to place the < 500 msec value in appropriate clinical context. However, values from individual tracings within triplicate measurements that are ≥ 500 msec will not be included in the categorical analyses unless the average from the triplicate measurements is also ≥ 500 msec.

Individual participant profiles will be created for those participants meeting the sponsor's abnormality criteria – including absolute values, change from baseline and percent change from baseline. Listings of participants with any single post dose value > 500 msec will also be produced for QTcF.

6.2.1.3. Vital sign measurements

Absolute values and changes from baseline in supine systolic and diastolic BP, and PR will be summarized by treatment group and timepoint, according to sponsor reporting standards. Tables will be paged by parameter.

Maximum decrease from baseline for supine systolic and diastolic blood pressures and maximum increase from baseline for supine pulse rate will be summarized by treatment group, according to sponsor reporting standards.

Maximum absolute values and changes from baseline for vital signs will also be summarized descriptively by treatment group using categories as defined in Appendix 1. Numbers and percentages of participants meeting the categorical criteria will be provided. All planned and unplanned post dose time points will be counted in these categorical summaries. All values meeting the criteria of potential clinical concern will be listed.

6.2.1.4. Clinical safety laboratory measurements.

Laboratory data will be listed and summarized by treatment group and overall, in accordance with the sponsor reporting standards.

6.2.2. *Baseline adjusted QTcF of moxifloxacin and placebo at Tmax of 3, 4, and 5 hours on Day 3.*

Change from baseline in QTcF will be analyzed by MMRM as described in Section 5.2.4 using ECG analysis set. All planned post-dose time points on Day 3 and 4 of each period will be included in the model. The least-squares (LS) means of change from baseline QTcF of moxifloxacin and placebo, as well as the difference between moxifloxacin and placebo, and the corresponding 90% CIs will be reported for each post-dose timepoint. The 96.7% CIs for mean differences between moxifloxacin and placebo at 3, 4, and 5-hr pose-dose will also be provided as the Bonferroni-adjusted two-sided 90% CIs.

A visualization displaying the model predicted mean difference in change from baseline QTcF between moxifloxacin and placebo and the corresponding 90% CIs will also be created.

The study will be deemed adequately sensitive to detect QT/QTc prolongation if any of the lower limits of the two-sided Bonferroni-adjusted 90% CI (96.7% CI) for mean differences between moxifloxacin and placebo at any of the 3, 4 and 5-hr pose-dose, respectively, are greater than 5 msec.

6.3. Other Endpoint(s)

6.3.1. *Baseline adjusted heart rate, PR interval, and QRS intervals of sisunatovir and placebo at each postdose time point.*

Absolute values and changes from baseline for the HR, PR interval, QT interval, and QRS intervals will be summarized by treatment group and timepoint as described in Section 6.2.1.2.

The difference of ECG parameters, including uncorrected QT, QTcF, QTcB, HR, PR, and QRS, between active drugs (sisunatovir and moxifloxacin) and placebo for each participant will be summarized by post-dose timepoints. The time matched mean difference and the corresponding 90% CIs will be reported for each post-dose dose timepoint. Plots of time matched mean difference between active drugs and placebo (Y-axis) against time post-dose (X-axis) will also be provided.

6.3.2. *Plasma sisunatovir parameters, including C_{max}, T_{max} and area under the plasma concentration-time profile from time 0 to 24 hours (AUC_{24}) post-dose on Day 3.*

The PK parameter listed in the Table 3 will be summarized descriptively by treatment.

Table 3. PK Parameters

Parameter	Summary Statistics
C_{max}	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%.
T_{max}	N, median, minimum, maximum.

AUC_{24}	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%.
AUC_{12}*	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%.

*To be comparable with Microsampling.

Box and whisker plots for individual subject parameters (AUC_{24} and C_{max}) will be presented and overlaid with geometric means.

Presentations for sisunatovir concentrations will include:

- a listing of all concentrations sorted by subject ID 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 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 postdose (based on the summary of concentrations by time postdose).
- mean concentrations time plots (on both linear and semi-log scales) against nominal time postdose (based on the summary of concentrations by time postdose).
- individual concentration time plots by treatment (on both linear and semi-log scales) against actual time postdose.
- individual concentration time plots by subject (on both linear and semi-log scales) against actual time postdose (there will be separate plots for each subject per scale).

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.

6.3.3. *Concentration of sisunatovir from paired samples obtained via microsampling compared to venous sampling.*

AUC_{12} and C_{max} derived from blood samples collected using the Tasso® M20 device adjusted for B/P ratio will be summarized for sisunatovir and will include the set of summary statistics as specified in Table 3. Summary statistics (N, geometric mean, geometric CV, median, arithmetic mean, CV, minimum, maximum) of the whole blood concentrations, plasma equivalent concentrations by microsampling using individual B/P ratio and plasma equivalent concentration by microsampling using population geometric mean B/P ratio at

nominal time of collection as defined in SoA of the protocol for each treatment arm will be calculated.

Furthermore, to determine correlation in concentrations derived from time matched Tasso® M20 microsampling (calculated using individual or population) and traditional venous plasma sampling, Bland-Altman plot analysis will be performed to evaluate the bias and SD of the bias between the mean differences and to estimate an agreement interval within a 95% confidence limit. Plots of the difference between the two samples (on Y-axis) as a function of the average of the two samples (on X-axis). These plots will be colored by participant in addition to by timepoint. In addition, a correlation plot of the plasma PK sample (on X-axis) and Tasso PK sample (on Y-axis) will be created including a line of equality Y = X. These results will be reported in a separate Clinical Bioanalytics summary report and may not be included in the CSR.

6.4. Subset Analyses

No subset analyses will be performed.

6.5. Baseline and Other Summaries and Analyses

6.5.1. Baseline Summaries

The demographical variables, height, weight, and BMI collected during screening will be summarized. Other data collected at screening that are used for inclusion/exclusion criteria, such as laboratory data, will be considered source data, and will not be required to be reported, unless otherwise noted.

6.5.2. Study Conduct and Participant Disposition

Participant evaluation groups will show participant disposition by treatment. Frequency counts and percentages will be supplied for participant discontinuation(s) by treatment. Data will be reported in accordance with the sponsor reporting standards.

6.5.3. Concomitant Medications and Nondrug Treatments

All concomitant medication(s) as well as non-drug treatment(s) will be reported according to current sponsor reporting standards.

6.6. Safety Summaries and Analyses

See Section 3.2.1.

7. INTERIM ANALYSES

No interim analysis will be conducted for this study.

7.1. Introduction

Not applicable

7.2. Interim Analyses and Summaries

Not applicable

8. REFERENCES

1. Garnett C., et al. Scientific white paper on concentration-QTc modeling. Journal of Pharmacokinet and Pharmacodyn December 2017; https://doi.org/10.1007/s10928-017-9558-5.

2. Garnett C., et al. Methodologies to characterize the QT/corrected QT interval in the presence of drug-induced heart rate changes or other autonomic effects. Am Heart J 163(6): 912-30.etrics 1972;28:519-31.

APPENDICES

Appendix 1. Categorical Classes for ECG and Vital Signs of Potential Clinical Concern

Categories for QTcF and QTcB

Absolute Maximum (msec)	< 450	≥ 450 and < 480	≥ 480 and < 500	≥ 500
Maximum Increase from Baseline (msec)	< 30	≥ 30 and < 60	≥ 60	

Categories for PR

Absolute Maximum (msec)	≥ 300	
Maximum Increase from Baseline (msec)	Baseline > 200 and max. increase ≥ 25%	Baseline ≤ 200 and max. increase ≥ 50%

Categories for QRS

Absolute Maximum (msec)	≥ 140
Maximum Increase from Baseline (msec)	max. increase ≥ 50%

Categories for HR

Absolute Maximum (bpm)	> 120
Absolute Minimum (bpm)	< 40

Categories 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
Supine pulse rate (bpm)	min. <40	max. >120

Appendix 2. Example SAS Code for MMRM Model

```
proc mixed data=ecg;
        class participant sequence period time treat;
        model qtcf=sequence period baseline time treat time*treat/ddfm=kr;
        repeated time/ subject=period* participant type=cs;
        lsmeans treat*time/cl diff alpha=0.1;
        lsmeans treat*time/cl diff alpha = 0.033;
run;
```

Appendix 3. List of Abbreviations

Abbreviation	Term
Abs	Absolute
AE	adverse event
AUC	area under the curve
AUC_{12}	area under the curve from time 0 to 12 hours
AUC_{24}	area under the curve from time 0 to 24 hours
BLQ	below the limit of quantitation
BP	blood pressure
BPM	beats per minute
B/P	blood to plasma
CI	confidence interval
C_{max}	maximum observed concentration
C-QT	concentration QT
CRU	clinical research unit
CSR	clinical study report
CV	coefficient of variation
ECG	electrocardiogram
HR	heart rate
ICH	International Council for Harmonisation
LLOQ	lower limit of quantitation
LS	least-squares
LSM	least-squares mean
MAR	missing at random
Min	minute
MMRM	mixed-effects model with repeated measures
Msec	millisecond
N/A	not applicable
ND	not done

Abbreviation	Term
NS	no sample
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PR	PR interval
PT	preferred term
Q&As	questions and answers
Q-Q	quantile-quantile
QRS	QRS complex
QTc	corrected QT
QTcB	corrected QT (Bazett's method)
QTcF	corrected QT (Fridericia method)
RR	RR interval
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
Sec	second
SoA	schedule of activities
TEAE	treatment-emergent adverse event
TQT	thorough QT
T_{max}	time for maximum observed concentration