Statistical Analysis Plan LOXO-BTK-20011

A Phase I, Single-Dose, Randomized, Partially Double-Blind, Placebo- and Positive-Controlled, 3-Way Crossover Study to Evaluate the Effect of LOXO-305 on QTc Interval in Healthy Subjects

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Protocol: LOXO-BTK-20011

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Final 1.0	22 February 2021		Final version.

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1 Abbreviations

Abbreviation	Term/Description
bpm	Beats per minute
CI	Confidence interval
C_{max}	Maximum plasma concentration
Δ	Change-from-baseline
ΔΔ	Placebo-corrected and placebo-adjusted change-from-baseline
ECG	Electrocardiogram
HR	Heart rate
LOESS	Locally weighted scatter plot smoothing
LS	Least squares
ms	Millisecond
PK	Pharmacokinetic(s)
PR	PR interval of the ECG
Q-Q	Quantile-quantile
QRS	QRS interval of the ECG
QT	QT interval of the ECG
QTc	Corrected QT interval
QTcF	Corrected QT interval using Fridericia's formula
QTcI	Optimized HR-corrected QT interval
QTcS	Individualized heart rate-corrected QT interval
RR	RR interval of the ECG
SAP	Statistical analysis plan
SD	Standard deviation
SE	Standard error
t_{max}	Time to reach C_{max}
TQT	Thorough QT

2 Introduction

This statistical analysis plan (SAP) was developed after review of the protocol LOXO-BTK-20011 (Protocol version 1.0 dated 30 November 2020) for the study "A Phase I, Single-Dose, Randomized, Partially Double-Blind, Placebo- and Positive-Controlled, 3-Way Crossover Study to Evaluate the Effect of LOXO-305 on QTc Interval in Healthy Adult Subjects" and the ERT contract/proposal. This document defines the populations to be analyzed and provides full details of the statistical analyses, data displays, and algorithms to be used for data derivations to aid in the production of the statistical output and the statistical section of the cardiac safety report in regard to electrocardiogram (ECG) and concentration-QTc analyses. Relevant subject characteristics as well as the electrocardiographic parameters that will be evaluated are described along with the specific statistical methods.

3 Study Design

This is a single-dose, randomized, partially double-blind (for LOXO-305 and placebo), placebo and positive-controlled, 3-way crossover design study.

All subjects will participate in 3 treatment periods (Treatment Periods 1, 2, and 3); in each treatment period, subjects will receive either a single oral dose of 900 mg LOXO-305 (Treatment A), a single oral dose of 900 mg LOXO-305 matched placebo (Treatment B), or a single oral dose of 400 mg moxifloxacin (Treatment C). Assignment to Treatment A or B will be blinded but Treatment C will be open label. All subjects will receive Treatments A, B, and C on 1 occasion during the study. There will be a washout period of at least 10 days between dosing in each period.

Subjects will be randomly assigned to 1 of 6 treatment sequences (e.g, ABC, BCA, CAB, ACB, CBA, and BAC), according to the randomization scheme issued by Covance. A total of 30 subjects will be randomized, such that 5 subjects will be assigned to each treatment sequence.

Each treatment (A, B, and C) will be administered orally in the mornings of Day 1, Day 12, and Day 23 following a fast of at least 8 hours prior to and 6 hours after dosing.

4 Cardiodynamic ECG Assessment

4.1 ECG and Pharmacokinetic Sample Collection

Holter monitors will be used to collect continuous 12-lead ECG data for the purpose of collecting cardiodynamic ECGs for approximately 25.5 hours, starting at approximately 1.5 hours prior to dosing and at least 24 hours post-dose. Recording will be started and stopped at logistically optimal times to ensure that all scheduled time points are collected. Up to 10 replicate 12-lead ECG recordings will be extracted from the continuous Holter recording at the scheduled time points, prior to the collection of blood samples for PK analysis.

The time points for ECG extraction in each period are 0.75, 0.5, and 0.25 hours prior to dosing on Day 1 and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8.5, 12, and 24 hours post-dose (the 24 hours post-dose time point will occur on Day 2, at the end of the Day 1 Holter recording).

Timing and recording technique for ECGs will be standardized for all subjects. Subjects must be awakened at least 1 hour prior to the start of the cardiodynamic ECGs on the day of dosing and before the ECG recording scheduled at the 24-hour post-dose time point.

Following dosing on days where cardiodynamic ECG assessments are performed, subjects will remain lying down or sitting and awake for the first 7 hours of the 24-hour post-dose cardiodynamic ECG monitoring period, as the QT-RR relationship is different during sleep. Subjects will be required to lie quietly in a supine position with minimal movement and minimal exposure to noise and other environmental stimuli (i.e., TV, loud radio, interactions with other subjects, etc.) for at least 10 minutes before and 5 minutes during the ECG extraction to allow for quality ECG extraction. In the event that an AE(s) occurs at any time during cardiodynamic ECG recordings, subjects may be placed in an appropriate position or will be permitted to lie down on their right side.

All ECG extraction should occur in a 5-minute time window around the scheduled/nominal time and always precede the PK draw. If targeted ECG time points are artefactual or of poor quality, analyzable 10-second ECGs will be extracted as close as possible to the targeted time points.

Blood samples for PK analysis of plasma concentrations of LOXO-305 or moxifloxacin plasma levels, and for future potential and/or exploratory analysis will be collected at the time points immediately after the ECG extractions.

All Holter/ECG data will be collected using M12R continuous 12-lead digital recorders and the M12A Enterprise Holter System Client (Global Instrumentation, LLC, Manlius, New York, USA). The equipment will be supplied and supported by ERT.

ECG intervals will be measured by the core laboratory in a blinded manner using the Early Precision QT technique (see Appendix A for more details). The ECG database will be locked before any statistical analysis is undertaken.

4.2 Study Objectives

4.2.1 Primary Objective

The primary ECG objective is to evaluate the effects of a supratherapeutic exposure of LOXO-305 on the QTc interval corrected for heart rate (HR) compared with placebo in healthy subjects.

4.2.2 Secondary Objectives

- To assess the effect of a single supratherapeutic dose exposure of LOXO-305 on other ECG parameters (HR, PR, and QRS interval) and on T-wave morphology changes compared with placebo in healthy subjects.

- To demonstrate sensitivity of the study to detect a small QTc effect using 400 mg oral moxifloxacin as a positive control in healthy subjects.
- To evaluate the PK of a single supratherapeutic dose of LOXO-305 in healthy subjects.
- To evaluate the safety and tolerability of a single supratherapeutic dose of LOXO-305 in healthy subjects.

4.3 Cardiodynamic ECG Endpoints

4.3.1 Primary Endpoint

The primary endpoint is the placebo-corrected change from baseline (ΔΔ) QTc interval (ΔΔQTc). The default will be ΔΔQTcF with QTc interval corrected for HR using the Fridericia method (QTcF).

If a substantial HR effect is observed (i.e., the largest least squares [LS] mean ΔΔHR is greater than 10 bpm in the by-time point analysis), other correction methods such as optimized HR-corrected QT interval (QTcI), and individual HR-corrected QT interval (QTcS) will be explored and compared. The method that removes the HR dependence of the QT interval most efficiently will be chosen as the primary correction method for this primary endpoint.

4.3.2 Secondary Endpoints

The secondary endpoints are:

- Change from baseline (Δ) QTcF, HR, PR, QRS intervals (ΔQTcF, ΔHR, ΔPR, and ΔQRS).
- If a substantial HR effect is observed: Change-from-baseline QTcS, and QTcI (ΔQTcS, and ΔQTcI).
- Placebo-corrected change from baseline HR, PR, and QRS (ΔΔHR, ΔΔPR, ΔΔQRS).
- If a substantial HR effect is observed: Change-from-baseline QTcS, and QTcI (ΔQTcS, and ΔQTcI).
- If a substantial HR effect is observed: Placebo-corrected ΔQTcF, and/or ΔQTcS, and/or ΔQTcI (ΔΔQTcF, ΔΔQTcS, ΔΔQTcI) if not selected as the primary endpoint.
- Categorical outliers for QTcF (and QTcS, and QTcI if a substantial HR effect is observed), HR, PR, and QRS.
- Frequency of treatment-emergent changes of T-wave morphology and U-wave presence.

5 Statistical Methods

5.1 General Methodology

All statistical analyses will be performed using the statistical software SAS for Windows Version 9.4 or higher (SAS Institute, Inc., Cary, NC). Data collected from all randomized subjects will be

presented in data listings. Both absolute values and change-from-baseline values for each subject will be given where applicable. All continuous data will be listed with the same precision as will be presented in the database. Data listings will be sorted by treatment, subject ID, and time point. Missing values will be represented by an empty cell and no imputation will be made.

For all descriptive statistics of continuous ECG parameters (i.e., HR, PR, QRS, and QTcF), data will be summarized including number of subjects (n), mean, median, standard deviation (SD), standard error (SE), 90% confidence interval (CI), minimum, and maximum by treatment and time point. For all modeling results of the by-time-point analysis of change-from-baseline values of continuous ECG parameters, n, least squares (LS) mean, SE, and 90% CI will be included. Modeling results of the by-time point analysis of placebo-corrected change-from-baseline will also include LS mean, SE, and 90% CI. Mean and median values will be rounded to the nearest tenth. SD, SE, and CI will be rounded to the nearest hundredth. For the concentration-QTc analysis, 2 decimal places will be shown for all effect estimates for all results which have an absolute value greater than 0.05. Each effect estimate with an absolute value ≤ 0.05 will be displayed with 2 significant figures. The CI of the effect estimate will display 1 more decimal place than the effect estimate. SE and *P* values will be reported with 4 digits and *P* values < 0.0001 will be reported as < 0.0001. Degrees of freedom (*df*), and t-value will be reported to the nearest tenth and nearest hundredth, respectively. Percentages will be rounded to the nearest tenths decimal place.

5.2 Analysis Populations (Subject-Level) and Record-Level Analysis Sets

The analysis populations (subject-level) for cardiodynamic ECG assessment are defined as follows (Table 1).

Table 1 Analysis populations (subject-level) for cardiodynamic ECG assessment

Population	Subjects
QT/QTc population	All subjects who have received 1 dose of study drug (LOXO-305, moxifloxacin, or placebo) with measurements at baseline as well as on-treatment with at least 1 post-dose time point with a valid ΔQTc value. The QT/QTc population will be used for the by-time point and categorical analyses of the cardiodynamic ECG parameters.
PK Population	All subjects who received 1 dose of LOXO-305 or moxifloxacin, and have at least 1 qauntifiable plasma concentration of LOXO-305 or moxifloxacin and have evaluable pharmacokinetic (PK) data. A subject may be excluded from the PK summary statistics and statistical analysis if the subject has an AE of vomiting that occurs at or before 2 times the median t_{max}. The impact of protocol deviations on the PK population will be evaluated on a case-by-case basis.
PK/QTc population	All subjects who are in both the PK and QT/QTc populations with at least 1 pair of post-dose PK and ΔQTc data from the same time point in at least 1 period as well as subjects in the QT/QTc population who received placebo.

Population	Subjects
	The PK/QT population will be used for the concentration-QTc analysis and assay sensitivity. The PK/QTc population will be defined for LOXO-305 and moxifloxacin.

Table 2 defines the observations that will be included at a record/time point level in the statistical analyses described in this SAP.

Table 2 Record-level analysis sets for cardiodynamic ECG assessment

Analysis set	Definition
By-time point analysis set	For all continuous ECG parameters, all post-baseline time points that have a nonmissing change-from-baseline observation. Defined for subjects in the QT/QTc analysis set.
Categorical analysis set	For all categorical ECG parameters, all post-baseline time points that have an evaluable record at baseline, i.e., treatment-emergent changes can be assessed. Defined for all subjects in the QT/QTc analysis set.
Concentration-QTc analysis set	All time points in the by-time point analysis set that have a corresponding planned PK collection at the same time point. Time points at which no PK data are available for nonplacebo subjects will not be retained. Defined for QTc for subjects in the PK/QTc analysis set.

5.3 Baseline

For all continuous ECG parameters, baseline will be the average of the derived ECG intervals from the 3 time points (0.75, 0.5, and 0.25 hours) prior to treatment administration for each period on Day 1, Day 12, and Day 23, respectively. For T-wave morphology and U-wave presence, baseline includes findings observed in any of the replicates from the 3 time points prior to dosing for each period on Day 1, Day 12, and Day 23, respectively.

5.4 QT Correction Methods

The QT and RR value for each beat will be used for HR correction.

The Fridericia's correction (QTcF) is defined as $QTcF\ (ms) = QT\ (ms)/[RR(ms)/1000]^{1/3}$.

In case a substantial HR effect is observed on-treatment with LOXO-305 (i.e., if the largest least squares [LS] mean $\Delta\Delta HR > 10$ bpm in the by-time point analysis), additional QT correction methods may be explored. Since Holter recordings are not collected on Day -1, prior to treatment, additional QT correction methods will be derived from Day 1 of placebo period during

both the time points of supine rest (QTcS) and from all evaluable QT/RR pairs in the 24-hour recording (QTcI). These data will be used to obtain the RR interval (HR) and QT data to enable derivations of QTcS and QTcI, as follows.

- An individualized HR-corrected QT interval (QTcS) will be calculated from QT/RR data obtained at supine resting time points on Day 1 of the placebo period. Based on QT/RR pairs from all subjects, the QTcS correction coefficient will be derived from a linear mixed-effects model: $\log(QT_{ij}) = \log(a_i) + b1_i \times \log(RR_{ij}) + b2_i \times \text{gender}_i + b3_i \times \log(RR_{ij}) \times \text{gender}_i$ with gender included as a fixed effect, and subject included as a random effect. The coefficient of log(RR) for each subject, $b_i = b1_i + b3_i \times \text{gender}$, will then be used to calculate QTcS for that subject as follows: $QTcS = QT/RR^{b_i}$.
- An optimized HR-corrected QT interval (QTcI) will be derived from a broader range of HRs by using all QT/RR data from the full 24-hour recording (all acceptable beats) on Day 1 of the placebo period. The QT/RR pairs from each subject will be used for that subject's individual correction coefficient, which will be derived from a linear regression model: $\log(QT_i) = \log(a_i) + b_i \times \log(RR'_i)$. The coefficient of log(RR′) for each subject, b_i, will then be used to calculate QTcI for that subject as follows: $QTcI = QT/RR'^{b_i}$.

RR′ will be derived using the unadjusted RR, 1-minute average RR, 3-minutes average RR, 1-minute weighted average RR and 3-minutes weighted average RR, respectively. Hysteresis adjustment will also be applied to the extracted ECGs with the same duration of averaging, and weighted averaging.

For QTcS and QTcI, the individual correction coefficients, b_i, will be listed and also summarized in a table using arithmetic mean, SE, number of subjects, and 90% CI (based on a *t*-distribution).

The method that removes the HR dependence of the QT interval most efficiently will be chosen as the primary correction method.

6 Analyses

6.1 Evaluation of QT/RR Correction Methods

In case a substantial HR effect is observed, defined as the largest LS mean ΔΔHR greater than 10 bpm, the relationship between QTc (QTcF and, if derived, QTcS [individual], QTcI [optimized] using unadjusted RR, 1-minute average RR, 3-minutes average RR, 1-minute weighted average RR and 3-minutes weighted average RR) and RR interval will be investigated using on-treatment data (LOXO-305, and moxifloxacin) by a linear regression model: $QTc = c + d \times RR$. Mean QTc and RR values from all nominal time points (including pre-dose) will be used. The RR coefficient for each subject, d_i, will then be used to calculate the average sum of squared slopes (SSS) for each of the different QT/RR correction methods. In addition, a scatter plot and quantile plot of QTc (QTcF, QTcS, QTcI) and RR intervals by treatment with a regression line and a linear mixed-effects line (90% CI), respectively, will also be given. QTcI based on a 3-minute weighted average will be used as the primary endpoint, with other corrections as supporting evidence.

6.2 Concentration-QTc Analysis (Primary Analysis)

The relationship between LOXO-305 plasma concentrations and change-from-baseline QTc (ΔQTcF, or ΔQTc corrected with the HR correction method chosen as primary if a substantial HR effect is observed) be quantified using a linear mixed-effects modeling approach. The model will include ΔQTc as the dependent variable, LOXO-305 plasma concentrations as the explanatory variate (0 for placebo), centered baseline QTc (i.e., baseline QTc for individual subject minus the population mean baseline QTc for all subjects in the same treatment period) as an additional covariate, treatment (active = 1 or placebo = 0), time (i.e., nominal post-dose time point) as fixed effects, and random effects on the intercept and slope per subject[1]. In addition, if there are significant concentrations of LOXO-305 metabolites, their concentrations/QTc relationship may be explored (exploratory metabolite profiling has shown that unchanged LOXO-305 is the major drug-related component in human plasma). In all calculations, zero will be substituted for concentrations below the quantification limit of the assay and for concentrations from subjects who received placebo.

An unstructured covariance matrix will be specified for the random effects. If convergence cannot be achieved even after appropriate rescaling of the concentrations, the random effect on the slope and intercept will be dropped, in this order, until convergence is achieved. The degrees of freedom of estimates will be determined by the Kenward-Roger method. From the model, the slope (i.e., the regression parameter for LOXO-305 concentrations) and the treatment effect-specific intercept (defined as the difference between active and placebo) will be estimated together with the 2-sided 90% CI. The estimates for the time effect will be reported with degrees of freedom and SE.

The geometric mean of the individual C_{max} values for subjects in the active dose group will be determined. The predicted effect and its 2-sided 90% CI for ΔΔQTc (i.e., slope estimate × concentration + treatment effect-specific intercept) at this geometric mean C_{max} of LOXO-305 will be obtained. If the upper bound of the 2-sided 90% CI of the predicted effect of ΔΔQTc at clinically relevant plasma levels of LOXO-305 is below 10 ms, it will be concluded that LOXO-305 does not cause clinically relevant QTc prolongation within the observed plasma concentration ranges.

To evaluate the adequacy of model fit with respect to the assumption of linearity, the observed ΔQTc values adjusted by population time effect estimated from the model will be used. These individual placebo-adjusted $\Delta QTc_{i,k}$ ($\Delta\Delta QTc_{i,k}$) values equal the observed individual $\Delta QTc_{i,k}$ for subject i administered with active drug or placebo at time point k minus the estimated population mean placebo effect at time point k (i.e., time effect). A quantile plot, i.e. plot of the quantiles (deciles) of observed drug concentrations and the mean placebo-adjusted ΔQTc (ΔΔQTc) and 90% CI at the median concentration within each decile will be given. The regression line presenting the model-predicted ΔΔQTc (as described by Tornøe et al[2]) will be added to evaluate the fit of a linear model and visualize the concentration-response relationship. Additional exploratory analyses (via graphical displays and/or model fitting) will include assessing for a delayed effect (hysteresis, Section 6.2.1) and the justification for the choice of the pharmacodynamic model (linear versus nonlinear, Section 6.2.2) as follows.

The SAS code for the concentration-QTc analysis using the full model is as follows.

```
PROC MIXED DATA=PKPD method=reml;
CLASS SUBJID TIME;
MODEL DQTc=TRT CONC TIME CBASE/ solution cl noint alpha=0.1 alphap=0.1 COVB DDFM=KR;
RANDOM INT CONC /type=UN SUBJECT=SUBJID s;
RUN;
```

Where PKPD = PK/QTc population, SUBJID = subject number, TRT = treatment (active = 1 or placebo = 0), TIME = nominal post-dose time point, CONC= time-matched plasma concentration of LOXO-305 (0 for placebo), CBASE = centered baseline QTcF, and DQTc = ΔQTcF.

Note: ESTIMATE statements will be included for the prediction of the effect in the active dose group.

6.2.1 Investigation of Hysteresis

Hysteresis will be assessed based on joint graphical displays of the least squares (LS) mean ΔΔQTc at each post-dose time point from the by-time point analysis and the mean concentrations of LOXO-305 at the same time points. In addition, hysteresis plots will be given for LS mean ΔΔQTc from the by-time point analysis and the mean concentrations. Other concentration-QTc models may be explored if both of the following conditions are met.

- A QT effect > 10 ms (i.e., LS mean ΔΔQTc > 10 ms) cannot be excluded in the by-time point analysis
- The difference (delay) between the time to reach the peak QTc effect (ΔΔQTc) and t_{max} is larger than 1 hour.

With the provision stated above, hysteresis will be assumed if the curve of the hysteresis plot shows a counterclockwise loop. A significant treatment effect-specific intercept may also be indicative of hysteresis or model misspecification, if it cannot be explained by a nonlinear relationship.

6.2.2 Appropriateness of a Linear Model

To assess the appropriateness of a linear model, normal quantile-quantile (Q-Q) plots for the standardized residuals and the random effects; scatter plots of standardized residuals versus concentration, fitted values, and centered baseline QTc; and box plots of standardized residuals versus nominal time, and active treatment will be produced, in addition to the quantile plot described above. Among these plots, the scatter plots of standardized residuals versus concentration and versus centered baseline QTc will also include the locally weighted scatter plot smoothing (LOESS) lines with optimal smoothing parameters selected by the Akaike information criterion with a correction (AICC)[3,4]. A scatter plot of observed concentration and ΔQTcF with a LOESS smooth line with 90% CI and a linear regression line will also be provided to check the assumption of a linear concentration-QTc relationship. If there is an indication that a linear model is inappropriate, additional models may be fitted, specifically an E_{max} model. The concentration-QTc analysis will then be repeated for the model found to best accommodate the nonlinearity detected.

6.3 Assay Sensitivity

The analysis to show assay sensitivity will be based on the concentration-QTc analysis of the effect on ΔQTc of 400 mg oral moxifloxacin using a similar model as for the primary analysis. That is, the relationship between moxifloxacin plasma concentration and ΔQTc will be investigated by linear mixed-effects modeling. The model will include ΔQTc as the dependent variable, moxifloxacin plasma concentration as the explanatory variable (0 for placebo), centered baseline QTc (i.e., baseline QTc for individual subject minus the population mean baseline QTc for all subjects in the same treatment period) as an additional covariate, study treatment (moxifloxacin = 1 or placebo = 0) and time (i.e., post-dose time point) as fixed effects, and a random intercept and slope per subject (Garnett et al[1]). The geometric mean of the individual C_{max} values for subjects receiving the single dose of 400 mg moxifloxacin will be determined. The predicted effect and its 2-sided 90% CI for ΔΔQTc (i.e., slope estimate × concentration + treatment effect-specific intercept) at this geometric mean C_{max} will be obtained.

If the slope of the moxifloxacin plasma concentration/ΔQTc relationship is statistically significant at the 10% level in a 2-sided test and the lower bound of the 2-sided 90% CI of the predicted QT effect at the observed geometric C_{max} of the 400 mg dose is above 5 ms, assay sensitivity will be deemed to have been demonstrated.

6.4 By-Time Point Analysis

The analysis for QTc will be based on a linear mixed-effects model with ΔQTc (ΔQTcF, or ΔQTc corrected per the HR correction method chosen as primary if a substantial HR effect is observed) as the dependent variable, period, sequence, time (i.e., nominal post-dose time point), treatment (LOXO-305, moxifloxacin and placebo), and time-by-treatment interaction as fixed effects, and baseline QTc as a covariate. An unstructured covariance matrix will be specified for the repeated measures at post-dose time points for subject within treatment period. If the model with unstructured covariance matrix fails to converge, other covariance matrix such as compound symmetry and autoregressive will be considered. The model will also include a subject-specific random effect. If the fixed effects for period and/or sequence should prove to be nonsignificant (that is, if the *P* value > 0.1), these effects may be removed from the model and the analysis will be repeated without those covariates. From this analysis, the LS mean, SE, and 2-sided 90 % CI will be calculated for the contrast "LOXO-305 versus placebo" at each post-dose time point, separately.

For HR, PR, QRS intervals, and QTc with the methods not selected as primary, the analysis will be based on the change-from-baseline post-dosing values (ΔHR, ΔPR, ΔQRS, and ΔQTc). The same (by-time point analysis) model will be used as described above for QTc. The LS mean, SE, and 2-sided 90% CI from the statistical modeling for both change-from-baseline and placebo-corrected change-from-baseline values will be listed in the tables and graphically displayed.

The SAS code for the by-time point analysis for QTc is as follows.

```
PROC MIXED DATA=ECG;
CLASS SUBJID TREAT TIME PERIOD SEQUENCE;
```

MODEL DQTc=BASE TREAT TIME TREAT*TIME PERIOD SEQUENCE/DDFM=KR;
RANDOM INTERCEPT / SUBJECT =SUBJID TYPE=UN;
REPEATED TIME / SUBJECT = PERIOD*SUBJID TYPE = UN;
LSMEANS TREAT*TIME/CL DIFF ALPHA=0.1;
RUN;

Where ECG = QT/QTc population, SUBJID = subject number, TREAT = treatment (LOXO-305, moxifloxacin and placebo), TIME = nominal post-dose time point, BASE = baseline QTc, PERIOD = period, SEQUENCE = sequence, and DQTc = ΔQTc.

6.5 Categorical Analysis

Results for categorical outliers will be summarized in frequency tables with counts and percentages for both number of subjects and number of time points. Subject data will be summarized using the count of distinct subjects that fall into the category and the percentage of the total number of subjects. Time point data will be summarized using the count of time points at which the assessments fall into the category and the percentage of the total number of time points at which assessments are performed. Counts (either number of subjects or number of time points) for each treatment group will be used as the denominator in the calculation of percentages unless otherwise specified.

A subject or time point will be determined as an outlier if the following criteria (which are assessed separately) are met for the ECG intervals (Table 3).

Table 3 Criteria for determining a subject or time point outlier

ECG interval	Categorical outlier criteria
QTc (QTcF, QTcS, QTcI)	Treatment-emergent value of > 450 and ≤ 480 ms when not present at baseline (new onset)
	Treatment-emergent value of > 480 and ≤ 500 ms when not present at baseline (new onset)
	Treatment-emergent value of > 500 ms when not present at baseline (new onset)
	Increase of QTc from baseline of > 30 and ≤ 60 ms
	Increase of QTc from baseline > 60 ms
PR	Increase of PR from baseline > 25% resulting in PR > 200 ms
QRS	Increase of QRS from baseline > 25% resulting in QRS > 120 ms
HR	Decrease of HR from baseline >25% resulting in HR < 50 bpm
	Increase of HR from baseline >25% resulting in HR > 100 bpm

All outliers will be summarized for each treatment on the basis of incidence rates. A subject will be counted only once for a particular outlier event if the subject experiences more than 1 episode

of that event. The total number of time points will be based on the number of observed time points across all subjects within a treatment group.

6.6 Morphological analysis for T wave and U wave

Morphological analyses will be performed with a focus on detecting changes in T-wave morphology and appearance of abnormal U waves. The analyses will evaluate change-from-baseline (i.e., treatment-emergent changes).

The analysis results for T-wave morphology and U-wave presence will be summarized in frequency tables with counts and percentages for both number of subjects and number of time points. The number and percentage of subjects in each treatment group having changes from baseline that represent the appearance of the morphological abnormality will be summarized. The total number of time points having a particular change event will be summarized in terms of number and percentage based on the number of observed time points across all subjects within a treatment group.

6.7 Terminology and Definitions: Placebo-corrected ΔQTcF and Placebo-adjusted ΔQTcF (ΔΔQTcF)

Change-from-baseline QTcF (ΔQTcF) will be used as the dependent variable in the concentration-QTc analysis and in the by-time point analysis.

By-time Point Analysis

Placebo-corrected ΔQTcF (ΔΔQTcF)

- In the by-time point analysis on the QTcF interval, LS mean, SE, and 2-sided 90% CI of ΔQTcF and ΔΔQTcF will be calculated for active dose group and moxifloxacin group as well as on placebo group for ΔQTcF at each post-dose time point.

Concentration-QTc Analysis

Placebo-corrected ΔQTcF (ΔΔQTcF)

- In the concentration-QTc analysis, the term placebo-corrected ΔQTcF (ΔΔQTcF) will be used for the model-predicted effect across concentrations on a population level.
- *Definition for the estimated placebo-corrected ΔQTcF (ΔΔQTcF)*: Model-predicted mean ΔQTcF in active dose group or moxifloxacin group minus model-predicted mean ΔQTcF in the placebo group, which equals slope estimate × concentration + treatment effect-specific intercept.
    - The term placebo-corrected ΔQTcF (ΔΔQTcF) will be used for the model-predicted effect on the QTcF interval in the concentration-QTc prediction table and the scatter plots for concentration-QTc model(s), quantile plots, and prediction plots, as described in Section 8.

Placebo-adjusted ΔQTcF (ΔΔQTcF)

- In the concentration-QTc analysis, the term placebo-adjusted ΔQTcF (ΔΔQTcF) will be used to illustrate the underlying data on both subject and population levels.
- *Definition for the estimated placebo-adjusted ΔQTcF on a subject level:* observed ΔQTcF for each subject (on active dose group or moxifloxacin group or placebo group) minus the estimated time effect (i.e., the model-predicted mean ΔQTcF in the placebo group).
    - This term will be used to illustrate the underlying data on a subject level in the scatter plot(s) for concentration-QTc model(s), as described in Section 8.
- *Definition for the estimated placebo-adjusted ΔQTcF term on a population level:* the average of individually estimated placebo-adjusted ΔQTcF values at the associated median plasma concentration within each concentration decile.
    - This term will be used to illustrate the underlying data on a population level in the quantile plot(s), as described in Section 8.

6.8 Determination of Sample Size

A total of up to 30 subjects (5 subjects per treatment sequence) will be enrolled to ensure at least 24 evaluable subjects completed all three treatment periods of the study. A sample size of 24 evaluable subjects will provide at least 90% power to exclude that LOXO-305 causes more than a 10-ms QTc effect at clinically relevant plasma levels, as shown by the upper bound of the 2-sided 90% CI of the model-predicted QTc effect (ΔΔQTc) at the observed geometric mean Cmax of LOXO-305 in the study. This power is estimated approximately using a paired t-test. The calculation assumes a 1-sided 5% significance level, a small underlying effect of LOXO-305 of 3 ms, and a standard deviation (SD) of the ΔQTc of 8 ms for both LOXO-305 and placebo. The concentration-QTc analysis method is supported by Darpo et al[5] and Ferber et al[6], and is consistent with the experiences from 25 recent TQT studies.

6.8.1 Determination of Sample Size for Assay Sensitivity

To demonstrate assay sensitivity with concentration-QTc analysis, it has to be shown that the ΔΔQTc of a single dose of 400 mg moxifloxacin exceeds 5 ms (ie, the lower bound of the 2-sided 90% CI of the predicted QTc effect [ΔΔQTc] should exceed 5 ms). In a similarly designed, recent crossover study with 24 healthy subjects (on-file data, ERT), the standard error (SE) for the prediction of the QT effect of moxifloxacin based on the concentration-QTc analysis was 1.24 ms. The within-subject SD of ΔQTcF in the referred study was 5.4 ms based on the by-timepoint analysis. If the effect of moxifloxacin is assumed to be 10 ms, the SE of 1.24 ms corresponds to an effect size of (10-5)/(1.24 ×sqrt[24])= 0.82, where the effect size is the effect assumed under the alternative hypothesis divided by the SD of the test variable. This value should be compared to the effect size of 0.62 required to guarantee a power of at least 90% in a paired t-test situation with a sample size of 24 evaluable subjects. In other words, based on this calculation, a power of at least 90% for 24 evaluable subjects will be obtained as long as the variability of the ΔQTc, as measured by its within-subject SD, does not exceed 7.1 ms (ie, 132% [= 0.82/0.62] of the 5.4 ms observed in the referred study assuming the ratio of effective sizes is

consistent with inverse ratio of within-subject SD). The number also agrees with recent recommendations of the FDA, which propose at least 20 subjects[7].

7 References

1. Garnett C, Bonate PL, Dang Q, Ferber G, Huang D, Liu J, et al. Scientific white paper on concentration-QTc modeling. [Published correction appears in *J Pharmacokinet Pharmacodyn*. 2018;45(3):399]. *J Pharmacokinet Pharmacodyn*. 2018;45(3):383-397.
2. Tornøe CW, Garnett CE, Wang Y, Florian J, Li M, Gobburu JV. Creation of a knowledge management system for QT analyses. *J Clin Pharmacol*. 2011;51(7):1035-1042.
3. Cleveland WS. Robust locally weighted regression and smoothing scatterplots. *J Am Stat Assoc*. 1979;74(368):829-836.
4. Hurvich CM, Simonoff JS, and Tsai CL. Smoothing parameter selection in nonparametric regression using an improved Akaike Information Criterion. *J R Stat Soc Series B Stat Methodol*. 1998;60(2):271-293.
5. Darpo B, Benson C, Dota C, et al. Results From the IQ-CSRC Prospective Study Support Replacement of the Thorough QT Study by QT Assessment in the Early Clinical Phase. *Clin Pharmacol Ther*. 2015;97(4):326-335.
6. Ferber G, Zhou M, and Darpo B. Detection of QTc Effects in Small Studies--Implications for Replacing the Thorough QT Study. *Ann Noninvasive Electrocardiol*. 2015;20(4):368-377.
7. Huang DP, Chen J, Dang Q, and Tsong Y: Assay sensitivity in "Hybrid thorough QT/QTc (TQT)" study, Journal of Biopharmaceutical Statistics, 2019; 29(2), 378-384.

8 Tables, Figures, and Listings

8.1 Tables

Number	Title	Comments
14.1.1	QTcS and QTcI individual correction coefficients with descriptive statistics	Number of subjects (n), mean, SD, SE, 90% CI, median, minimum, and maximum will be given (Section 5.4).
14.1.2	Average SSS for different QT-RR correction methods	SSS for LOXO-305, moxifloxacin, and placebo will be given (Section 6.1).
14.1.3	Baseline values of ECG parameters with descriptive statistics	Number of subjects (n), mean, SD, SE, 90% CI, median, minimum, and maximum from descriptive analysis will be given by treatment for each ECG parameter (Section 6.4).
14.1.4	Observed values of QTc (primary correction method) with descriptive statistics	n, mean, SD, SE, 90% CI, median, minimum, and maximum from descriptive statistics will be given by treatment and post-dose time point (Section 6.4).

Number	Title	Comments
14.1.5.1-14.1.5.10	Change-from-baseline QTcF (and QTcI in 5 ways and QTcS if calculated), HR, PR, and QRS (ΔQTcF, ΔQTcI, ΔQTcS, ΔHR, ΔPR, and ΔQRS) at each time point	n, LS mean, SE, and 90% CI from the statistical modeling will be given by treatment and time point (Section 6.4).
14.1.6.1-14.1.6.10	Placebo-corrected change-from-baseline QTcF (and QTcI in 5 ways and QTcS if calculated), HR, PR, and QRS (ΔΔQTcF, ΔΔQTcI, ΔΔQTcS, ΔΔHR, ΔΔPR, and ΔΔQRS) at each time point	LS mean, SE, and 90% CI from the statistical modeling will be given by treatment and time point (Section 6.4).
14.1.7	QTc outliers per absolute category	Number (%) of subjects and time points with QTc > 450 and ≤ 480 ms, > 480 and ≤ 500 ms, or > 500 ms by treatment (Section 6.5).
14.1.8	QTc outliers per change-from-baseline category	Number (%) of subjects and time points with ΔQTc > 30 and ≤ 60 ms, or > 60 ms by treatment (Section 6.5).
14.1.9	Categorical analyses for HR, PR, and QRS	Number (%) of subjects and time points with ΔPR > 25% and PR > 200 ms at post-dose; ΔQRS > 25% and QRS > 120 ms at post-dose; HR decrease from baseline > 25% and HR < 50 bpm at post-dose; and HR increase from baseline > 25% and HR > 100 bpm at post-dose (Section 6.5).
14.1.10	T-wave morphology and U-wave presence across treatment groups: treatment-emergent changes	Number (%) of subjects and time points falling into each of the T-wave and U-wave categories will be given by treatment (Section 6.6).
14.1.11.1	Concentration-QTc analysis of LOXO-305 and associated ΔQTc prolongation	Fixed-effect estimations and corresponding *P* values will be given (Section 6.2).
14.1.11.2	Assay sensitivity analysis of moxifloxacin and associated ΔQTc prolongation	Fixed effect estimations and corresponding *P* values will be given (Section 6.3).
14.1.12.1	Predicted ΔΔQTc interval at geometric mean peak LOXO-305 concentration	Section 6.2. Referred to above as a "Concentration-QTc prediction table"
14.1.12.2	Predicted ΔΔQTc interval at geometric mean peak moxifloxacin concentration	Section 6.3. Referred to above as a "Concentration-QTc prediction table"

8.2 Figures

Number	Title	Comments
14.2.1.1	Scatter plots of QTc and RR by treatment	Scatter plots of QTc (QTcF, QTcIS, and QTcIS) and RR intervals by treatment with regression lines will be given (Section 6.1).
14.2.1.2	QTc-RR quantile plot by treatment	QTc-RR quantile plots (with quantiles) with linear mixed-effects line and 90% CI will be given (Section 6.1).
14.2.2	Observed QTcF (primary correction method) across time points	Mean and 90% CI from descriptive analysis will be given by treatment (Section 6.4).
14.2.3.1-14.2.3.10	Change-from-baseline QTcF (and QTcI in 5 ways and QTcS if calculated), HR, PR, and QRS (ΔQTcF, ΔQTcI, ΔQTcS, ΔHR, ΔPR, and ΔQRS) across time point	LS mean and 90% CI from the statistical modeling will be shown by treatment (Section 6.4).
14.2.4.1-14.2.4.10	Placebo-corrected change-from-baseline QTcF (and QTcI in 5 ways and QTcS), HR, PR, and QRS (ΔΔQTcF, ΔΔQTcI, ΔΔQTcS, ΔΔHR, ΔΔPR, and ΔΔQRS) across time point	LS mean and 90% CI from the statistical modeling will be shown by treatment (Section 6.4).
14.2.5.1	Mean LOXO-305 plasma concentrations over time	Section 6.2.
14.2.5.2	Mean moxifloxacin plasma concentration over time	Section 6.3.
14.2.6	LOXO-305 plasma concentrations and ΔΔQTc over time	Section 6.2.1.
14.2.7	Hysteresis plot of LOXO-305 plasma concentration and ΔΔQTc connected in temporal order	Section 6.2.1.
14.2.8.1	Scatter plot of observed LOXO-305 plasma concentrations and ΔQTc with simple linear regression line and LOESS regression	Scatter plot of ΔQTc versus concentration with LOESS line and 90% CI and simple regression line (Section 6.2.2).

Number	Title	Comments
14.2.8.2	Scatter plot of observed moxifloxacin plasma concentrations and ΔQTc with simple linear regression line and LOESS regression	Scatter plot of ΔQTc versus concentration with LOESS line and 90% CI and simple regression line (Section 6.3).
14.2.9.1	Scatter plot of observed LOXO-305 plasma concentrations and estimated placebo-adjusted ΔQTc	Scatter plot of placebo-adjusted ΔQTc versus concentration with linear mixed-effects regression line and 90% CI of ΔΔQTc (Section 6.2). Referred to above as "Scatter plot for concentration-QTc model(s)."
14.2.9.2	Scatter plot of observed plasma concentrations of moxifloxacin and estimated placebo-adjusted ΔQTc	Scatter plot of placebo-adjusted ΔQTc versus concentration with linear mixed-effects regression line and 90% CI of ΔΔQTc (Section 6.3). Referred to above as "Scatter plot for concentration-QTc model(s)."
14.2.10.1	Model-predicted ΔΔQTc (mean and 90% CI) and estimated placebo-adjusted ΔQTc (mean and 90% CI) across deciles of LOXO-305 plasma concentrations	Section 6.2. Referred to above as "Quantile plots."
14.2.10.2	Model-predicted ΔΔQTc (mean and 90% CI) and estimated placebo-adjusted ΔQTc (mean and 90% CI) across deciles of moxifloxacin plasma concentrations	Section 6.3. Referred to above as "Quantile plots."
14.2.11.1	Model-predicted ΔΔQTc interval at geometric mean peak LOXO-305 concentrations	Section 6.2. Referred to above as "Prediction plots."
14.2.11.2	Model-predicted ΔΔQTc interval (mean and 90% CI) at geometric mean peak moxifloxacin concentrations	Section 6.3. Referred to above as "Prediction plots."
14.2.12.1	Scatter plot of standardized residuals versus fitted values for LOXO-305	Section 6.2.2.
14.2.12.2	Scatter plot of standardized residuals versus fitted values for moxifloxacin	Section 6.3.

Number	Title	Comments
14.2.13.1	Scatter plot of standardized residuals versus concentrations with LOESS for LOXO-305	Section 6.2.2.
14.2.13.2	Scatter plot of standardized residuals versus concentrations with LOESS for moxifloxacin	Section 6.3.
14.2.14.1	Scatter plot of standardized residuals versus centered baseline QTc with LOESS for LOXO-305	Section 6.2.2.
14.2.14.2	Scatter plot of standardized residuals versus centered baseline QTc with LOESS for moxifloxacin	Section 6.3.
14.2.15.1	Box plot of standardized residuals versus nominal time for LOXO-305	Section 6.2.2.
14.2.15.2	Box plot of standardized residuals versus nominal time for moxifloxacin	Section 6.3.
14.2.16.1	Box plot of standardized residuals versus treatment for LOXO-305	Section 6.2.2.
14.2.16.2	Box plot of standardized residuals versus treatment for moxifloxacin	Section 6.3.
14.2.17.1	Normal Q-Q plot of standardized residuals for LOXO-305	Section 6.2.2.
14.2.17.2	Normal Q-Q plot of standardized residuals for moxifloxacin	Section 6.3.
14.2.18.1	Normal Q-Q plots of the estimated random effects for LOXO-305	Section 6.2.2.
14.2.18.2	Normal Q-Q plots of the estimated random effects for moxifloxacin	Section 6.3.

8.3 Listings

Number	Title	Comments
16.2.1.1-16.2.1.10	QTcF (and QTcI in 5 ways and QTcS if calculated), HR, PR, and QRS intervals - observed and change-from-baseline values as well as categorical outliers	Sections 6.4 and 6.5.
16.2.2	T-wave morphology and U-wave presence	Section 6.6.
16.2.3	ΔQTc and time-matched LOXO-305 and moxifloxacin concentrations for each subject	Data for concentration-QTc analysis (Section 6.2) and assay sensitivity (Section 6.3).

9 Approvals

ERT

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02-Mar-21 | 07:50:25 GMT

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01-Mar-21 | 22:26:47 GMT

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Appendix A: Early Precision QT Analysis

Twelve-lead ECGs will be extracted in up to 10 replicates from each nominal time point pre-specified in the protocol. The median value of each parameter from the set of evaluable beats in each extracted replicate will be calculated, and then the mean of all available medians (minimum 3 medians) from the nominal time point will be used as the subject's reportable value at that time point.

Early Precision QT analysis (formerly High Precision QT analysis) will be performed on all analyzable (non-artifact) beats in the 10 ECG replicates (1 replicate consists of one 14 second ECG). Statistical quality control procedures will be used to review and assess all beats and identify "high" and "low" confidence beats using several criteria including:

- QT or QTc values exceeding or below certain thresholds (biologically unlikely)
- RR values exceeding or below certain thresholds (biologically unlikely)
- Rapid changes in QT, QTc, or RR from beat to beat

Placement of fiducials and measurements of all primary ECG parameters (QT, QTc, RR) in all recorded beats of all replicates will be performed using the iCOMPAS software. All beats that are deemed "high confidence" will not be reviewed by an ERT ECG analyst. All low confidence beats will be reviewed manually by an ERT ECG analyst and adjudicated using pass-fail criteria. The beats found acceptable by manual review will be included in the analysis. The beats confirmed to meet fail criteria will not be included in the analysis.

For the purpose of measuring PR and QRS intervals and to assess T-wave morphology and presence of U-waves, the TQT Plus algorithm will select the 3 ECG replicates with the highest quality score from the ECG extraction window. These 3 ECGs will be analyzed using a semi-automated process to determine these parameters. If 3 consecutive usable beats cannot be identified in at least 2 of the 3 replicates, then all beats in all replicates will be reviewed for that time point using a manual analysis.

If manual analysis is required, then all beats in a minimum of 3 replicates will be reviewed using the iCOMPAS software. The ERT ECG analyst will review all usable beats in Lead II (or an alternate lead) for each replicate and will review and/or adjust the fiducial placements (onset of *P*, onset of Q, offset of S, and offset of T-wave that were electronically marked) of each waveform and also document the T-wave morphology and the presence of U-waves for each beat. A replicate will only be reported if it has 3 approved, usable beats.