Statistical Analysis Plan

Single-dose and Randomized, Single-center, Placebo- and Active-controlled, Crossover Study to Assess the Effect of Omecamtiv Mecarbil (OM) on QT/QTc Intervals in Healthy Subjects

Protocol: 20090231

Sponsor: Amgen Inc.

ERT Internal ID: 287-1001

Version: Final 1.0
Authors: [redacted], PhD, Principal Biostatistician
Date: 15 April 2020

NCT Number: NCT04175808
This NCT number has been applied to the document for purposes of posting on Clinicaltrials.gov

Revision History

Version	Issue Date	Author(s)	Description
Draft 0.1	02 January 2020	[redacted]	Initial version for review.
Draft 0.2	09 March 2020		Revised version.
Draft 0.3	27 March 2020		Revised version.
Draft 0.4	07 April 2020		Revised version.
Final 1.0	15 April 2020		Final version.

Table of Contents

1 Abbreviations

Abbreviation	Term/Description
ADaM	Analysis Data Model
bpm	Beats per minute
CDISC	Clinical Data Interchange Standards Consortium
CI	Confidence interval
C_{max}	Maximum plasma concentration
df	Degree of freedom
Δ	Change-from-baseline
ΔΔ	Placebo-corrected change-from-baseline
ECG	Electrocardiogram
E_{max}	Maximum effect model
FAS	Full analysis set
HR	Heart rate
LOESS	Locally weighted scatter plot smoothing
LS	Least squares
ms	Millisecond
n	Number of subjects.
OM	Omecamtiv mecarbil
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
RR	RR interval of the ECG
SAP	Statistical analysis plan
SD	Standard deviation
SE	Standard error
TQT	Thorough QT

2 Introduction

This statistical analysis plan (SAP) was developed after review of the protocol 20090231 (Version 4.0, Amendment 3 dated 23 August 2019) for the study "Single-dose and Randomized, Single-center, Placebo- and Active-controlled, Crossover Study to Assess the Effect of Omecamtiv Mecarbil (OM) on QT/QTc Intervals in Healthy 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

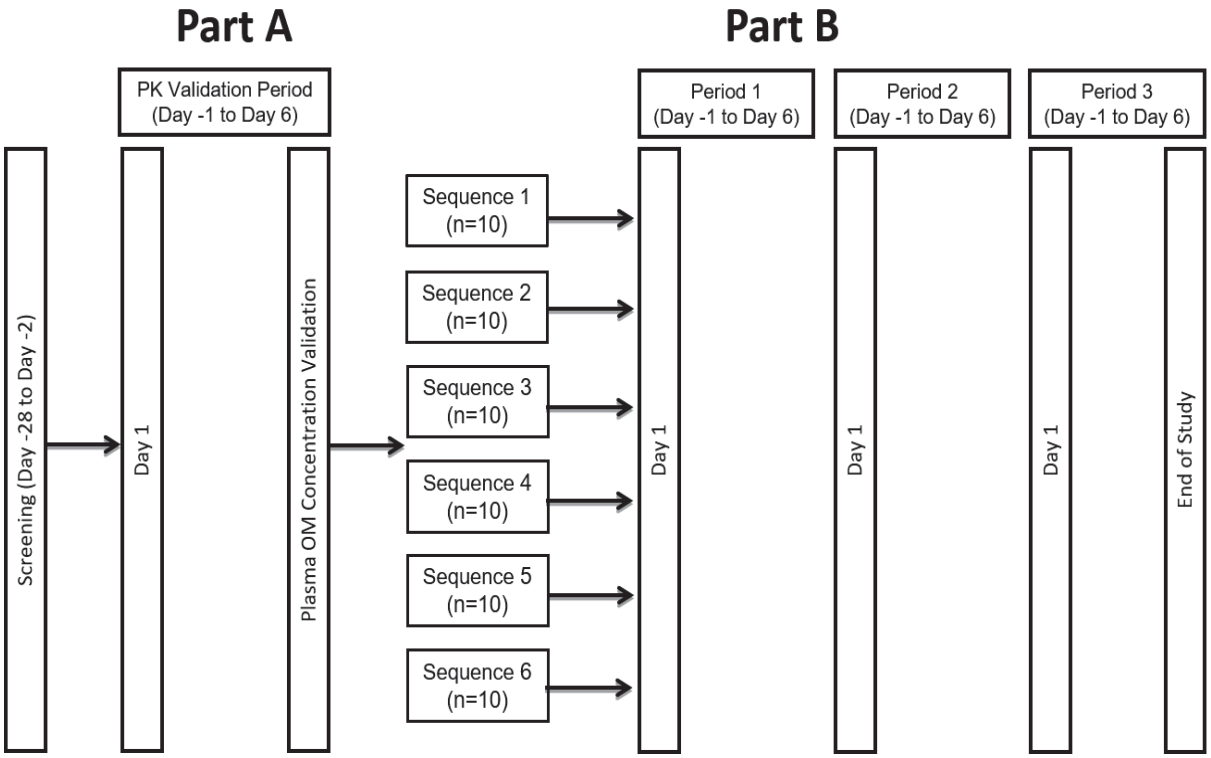
This will be a single-dose and randomized, single-center, 3-period, 3-treatment crossover study in healthy adult subjects to determine whether omecamtiv mecarbil (OM) prolongs the QT/QTc interval after a single oral dose administration of 50 mg OM. The total duration of participation in the study will be approximately 55 days, including the screening period. The study will be conducted at a single clinical site.

An overview of the study design is shown in Figure 1.

Approximately 60 to 70 subjects will be enrolled in Part A in order to have 60 subjects (10 per sequence) enrolled in Part B. With approximately 60 subjects in Part B, it is estimated that approximately 48 evaluable subjects will have data from all 3 periods in Part B.

Treatment in Part A will consist of a single 25-mg OM oral dose (Treatment 1), after which subjects whose resulting C_{max} is $\leq$350 ng/mL will be randomized into Part B. Part B consists of 3 periods, in which a single treatment is given in each period. Treatments in Part B will consist of placebo oral solution (Treatment A), OM 50 mg oral solution (Treatment B), and moxifloxacin 400 mg (Treatment C). All study treatments (Part A and Part B/Period 1, Period 2, and Period 3) will be separated by a washout of at least 7 days.

Figure 1 Study schematic



Note: All study treatments will be separated by a washout period of at least 7 days (including between Part A and Part B).

Part A

- Treatment 1: 25-mg OM oral solution

Subjects will be admitted to the research facility on Day −1 in Part A, at which time baseline procedures will be performed. After an overnight fast of at least 10 hours, subjects will be administered treatment with 250 mL of water. No food will be allowed for at least 4 hours postdose. Water is allowed as desired except for 1 hour before and after drug administration.

Subjects will stay at the research facility until Day 3. Pharmacokinetic (PK) samples will be collected to determine plasma OM concentrations. Subjects will be discharged after completion of Day 3 activities and will return for a safety follow-up visit on Day 6. PK results from Part A will be available prior to Part B. For subjects whose resulting C_{max} in Part A is > 350 ng/mL, the Day 6 visit will be considered their end of study visit.

Part B

- Treatment A: Placebo oral solution
- Treatment B: 50-mg OM oral solution
- Treatment C: 400-mg moxifloxacin oral tablet

After completing Part A, eligible subjects will be randomized to receive 3 treatments in 1 of 6 sequences, as follows:

Sequence	Period 1	Period 2	Period 3
1	A	B	C
2	B	C	A
3	C	A	B
4	A	C	B
5	C	B	A
6	B	A	C

Subjects will be admitted to the research facility on Day −1 of Period 1 in Part B. After an overnight fast of at least 10 hours, subjects will be administered treatment with 250 mL of water. No food will be allowed for at least 4 hours postdose. Water is allowed as desired except for 1 hour before and after drug administration. Placebo and OM treatments will be double-blinded and moxifloxacin treatment will be open-label. Subjects will receive standardized meals at approximately the same time.

Subjects will stay at the research facility throughout Part B, and study assessments will be performed according to the protocol, including continuous ECG recording, digital ECG extractions, and PK sample collections to determine plasma OM and moxifloxacin concentrations. Additionally, standard safety assessments, including 12-lead ECGs and vital signs monitoring, will be performed and safety and tolerability monitoring will be conducted throughout the study. Subjects will be discharged after completion of Day 6 activities in Period 3.

All study treatments will be separated by a washout period of at least 7 days (including between Part A and Part B).

4 Cardiodynamic ECG Assessment

4.1 ECG and PK Sample Collection

In Part B, continuous 12-lead digital ECG recording will be performed on Day 1 of each treatment period. The recording will be performed for approximately 27 hours in total. Subjects should lie down in a quiet room for at least 10 minutes before and 5 minutes after each extraction at the time points listed. Digital 12-lead ECGs will be extracted in up to 10 replicates at −1.25, −1, and −0.75 hours predose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 8, 12, 24 hours postdose.

The Investigator site will utilize their Mortara Surveyor system. The continuous 12-lead digital ECG data will be stored onto SD memory cards. The ECGs to be used in the analyses will be read centrally by ERT.

The following principles will be followed in ERT's core laboratory:

- ECG analysts are blinded to the subject, visit, and treatment allocation.

- Baseline and on-treatment ECGs for a particular subject will be over-read on the same lead and will be analyzed by the same reader.

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

Blood samples for PK determination will be drawn at the same time points as ECGs (i.e., predose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 8, 12, 24 hours postdose) and additionally at 48, 72, 96, 120 hours postdose. PK samples should be collected within 5 minutes after the completion of the digital ECG extraction window through 24 hours postdose and within ± 2 hours of the digital ECG extraction windows after 24 hours postdose.

4.2 Study Objectives

4.2.1 Primary Objective

The primary objective of the study is to assess the effect of a single therapeutic (50 mg) oral dose of OM on the QT/QTc interval, relative to placebo, in healthy subjects.

4.2.2 Secondary Objectives

The secondary objectives are:

- To assess the PK of OM after a single oral dose to healthy subjects.
- To evaluate the effect of OM on other ECG parameters (heart rate [HR], PR and QRS intervals, and treatment-emergent T-wave abnormalities and presence of U waves).
- To determine the plasma concentration-effect relationship for OM on the QT/QTc interval in healthy subjects.
- To evaluate assay sensitivity by evaluation of the positive control, a single 400-mg oral dose of moxifloxacin, on the QT/QTc interval in healthy subjects.
- To evaluate the safety and tolerability of OM after a single oral dose to healthy subjects.

4.3 Cardiodynamic ECG and Endpoints

4.3.1 Primary ECG Endpoint

The primary endpoint is the placebo-corrected change from baseline in QT interval corrected for HR based on the Fridericia correction (QTcF) interval (ΔΔQTcF) after OM dosing.

4.3.2 Secondary ECG Endpoints

The secondary endpoints are:

- ΔΔQTcF after moxifloxacin dosing
- Change-from-baseline HR, QTcF, PR and QRS intervals (ΔHR, ΔQTcF, ΔPR, and ΔQRS) after OM dosing

- Concentration-QTc analysis based on ΔΔQTcF after OM dosing
- Placebo-corrected ΔHR, ΔPR, and ΔQRS (ΔΔHR, ΔΔPR, and ΔΔQRS) after OM dosing
- Categorical outliers for QTcF, HR, PR, and QRS after OM dosing
- Frequency of treatment-emergent changes in T-wave morphology and U-wave presence after OM dosing

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 observed 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 subject ID, treatment, and time point. Missing values will be represented by an empty cell and no imputation will be made unless specifically stated otherwise.

Analysis Data Model (ADaM) datasets will be prepared using Clinical Data Interchange Standards Consortium (CDISC) ADaM Version 2.1 and CDISC ADaM Implementation Guide Version 1.1. Pinnacle 21 Community Validator Version 2.2.0 will be utilized to ensure compliance with CDISC standards.

Continuous data will be summarized using descriptive statistics 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. Mean and median values will be rounded to the nearest tenth, or to the first non-zero decimal. SD, SE, and CI will be rounded to the nearest hundredth, or to 1 digit more than the nearest non-zero digit. For the concentration-QTc analysis, 3 significant digits will be kept for the effect estimates. *P* values will be reported with 4 digits and *P* values less than 0.0001 will be reported as < 0.0001. Percentages will be rounded up or down to the nearest tenths decimal place.

5.2 Analysis Populations

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

Table 1 Analysis sets for cardiodynamic ECG assessment in Part B

Population	Definition
Full analysis set (FAS)	All randomized subjects who have received at least 1 dose of each study treatment (OM, moxifloxacin, and placebo).
PK analysis set	All subjects who received at least 1 dose of OM and have evaluable PK data. Any subject who experiences emesis within 4 hours of dosing or diarrhea within 24 hours of dosing may be excluded from the PK analysis.

Population	Definition
QT/QTc analysis set	All subjects in FAS with measurements at baseline as well as on-treatment with at least 1 postdose time point with a valid ΔQTcF value. The QT/QTc analysis set will be used for the by-time point and categorical analyses of cardiodynamic ECG parameters.
PK/QTc analysis set	All subjects who are in both the QT/QTc and PK analysis sets with at least 1 pair of postdose PK and QTcF data from the same time point as well as subjects in the QT/QTc analysis set who received placebo. The PK/QTc analysis set will be used for the concentration-QTc analysis in Part B.

5.3 Baseline

For all continuous ECG parameters from each period, baseline will be the average of the measured ECG intervals from the 3 predose time points (−1.25, −1, and −0.75 hours) on Day 1 for the respective period. For T-wave morphology and U-wave presence, baseline includes findings observed in any of the replicates from the 3 predose time points (−45, −30, and −15 minutes) on Day 1 for the respective period.

5.4 QT Correction Methods

The QT and RR value for each beat will be used for HR correction. Twelve-lead ECGs will be extracted in up to 10 replicates from each nominal time point prespecified 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.

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

For evaluation of the HR-corrected QT interval, a scatter plot and quantile plot of QTcF and RR intervals by treatment with a regression line and a linear mixed-effects line (90% CI), respectively, also will be given.

6 Analysis

6.1 By-Time Point Analysis (Primary Analysis)

The primary analysis of the QT/QTc data will be the "by-time point" analysis for QTcF, based on a linear mixed-effects model with ΔQTcF as the dependent variable; period, sequence, time (categorical), treatment (OM, moxifloxacin, and placebo), and time-by-treatment interaction as fixed effects; and baseline QTcF as a covariate. An unstructured covariance matrix will be specified for the repeated measures at time points for subject within treatment period. If the model with an unstructured covariance matrix fails to converge, other covariance matrices 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 not significant (i.e., 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 least squares (LS) mean, SE, and 2-sided 90% CI will be calculated for the contrast "OM versus placebo" at each postdose time point, separately. If the upper bound of the CI of ΔΔQTcF lies below 10 ms for all postdose time points, OM will be concluded not to have a significant effect on QT interval prolongation.

The SAS code for the by-time point analysis for QTcF 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 analysis set, SUBJID=subject identifier, TREAT=treatment (OM, moxifloxacin, and placebo), TIME=nominal time point, BASE= baseline QTcF, PERIOD=period, SEQUENCE=sequence, and DQTC=ΔQTcF.

6.1.1 Secondary By-time Point Analysis

For HR, PR, and QRS interval, the analysis will be based on the change from baseline post-dosing (ΔHR, ΔPR, and ΔQRS). The same model will be used as described for QTcF in the by-time point analysis. 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 tables and graphically displayed.

6.1.2 Assay Sensitivity

The analysis to show assay sensitivity will be based on ΔQTcF of moxifloxacin. The same model will be used as described for the primary analysis.

For the time points 2, 3, and 4 hours after dose administration, the contrast in treatment ΔΔQTcF = "moxifloxacin – placebo" will be tested against the 1-sided null hypothesis ΔΔQTcF $\leq$ 5 ms at the 5% significance level. Multiplicity will be controlled using the Hochberg procedure.[1] If after this procedure the lower bound of the 2-sided 90% CI for LS mean ΔΔQTcF is significantly larger than 5 ms for at least 1 time point of these 3 time points, assay sensitivity will be considered shown. In addition, 2-sided 90% CIs will be obtained for the contrast at all time points and used in the figures.

6.2 Categorical Analysis (Secondary Analysis)

Results for categorical outliers, 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. 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 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 2).

Table 2 Criteria for determining a subject or time point outlier

ECG interval	Categorical outlier criteria
QTcF	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 QTcF from baseline of > 30 and ≤ 60 ms
	Increase of QTcF 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.

For T-wave morphology and U-wave presence, treatment-emergent changes will be assessed, i.e., changes not present at baseline. For each category of T-wave morphology and of U waves, the category will be deemed as present if observed in any replicates at the time point.

The T-wave morphology and U-wave presence categories are described as follows (Table 3).

Table 3 T-wave morphology and U-wave presence categories (assessed manually)

Category	Description
Normal T-wave (+)	Any positive T-wave not meeting any criterion below.
Flat T-wave	T-amplitude < 1 mm (either positive or negative), including flat isoelectric line.
Notched T-wave (+)	Presence of notch(es) of at least 0.05 mV amplitude on ascending or descending arm of the positive T-wave.
Biphasic	T-wave that contains a second component with an opposite phase that is at least 0.1 mV deep (both positive/negative and negative/positive and polyphasic T-waves included).
Normal T-wave (-)	T-amplitude that is negative, without biphasic T-wave or notches.
Notched T-wave (-)	Presence of notch(es) of at least 0.05 mV amplitude on descending or ascending arm of the negative T-wave.
U waves	Presence of abnormal U waves.

The number and percentage of subjects in each treatment 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 category will be summarized in terms of number and percentage based on the number of observed time points across all subjects within a treatment.

6.3 Concentration-QTc Analysis (Secondary Analysis)

The relationship between OM plasma concentration and ΔQTcF will be investigated by linear mixed-effects modeling with ΔQTcF as the dependent variable, time-matched concentration of OM as the explanatory variable (0 for placebo), centered baseline QTcF (i.e., baseline QTcF for individual subject minus the population mean baseline QTcF for all subjects in the same period) as an additional covariate, study treatment (OM = 1 or placebo = 0) and time (i.e., postdose time point) as fixed effects, and a random intercept and slope per subject (Garnett et al[2]). In all calculations, concentrations in subjects who received placebo will be set to zero. OM plasma concentrations below the quantifiable limit at predose will be set to zero and after dosing will be set to 1/2 the lower limit of quantitation in the concentration-QTc analysis.

An unstructured covariance matrix will be specified for the random effects. If the model with an unstructured covariance matrix fails to converge 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. If an unstructured covariance structure does not converge after rescaling and removing the random terms in the model, then other covariance matrices, such as compound symmetry and

autoregressive, will be considered until convergence is achieved. The degrees of freedom (*df)* estimates will be determined by the Kenward-Roger method. From the model, the slope (i.e., the regression parameter for the concentration) 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 effects will be reported with degrees of freedom and SE.

The geometric mean of the individual C_{max} values for subjects on the active dose group will be determined. The predicted population average ΔΔQTcF (ie, slope estimate × concentration + treatment effect-specific intercept) and its corresponding 2-sided 90% CI at this geometric mean C_{max} will be obtained.

The plot of the observed median-quantile OM concentrations and associated mean placebo-adjusted ΔQTcF (i.e., ΔΔQTcF) with 2-sided 90% CI together with the regression line presenting the predicted ΔΔQTcF (2-sided 90% CI; as described by Tornøe et al)[3] will be used to evaluate the adequacy of the model fit to the assumption of linearity and the impact on quantifying the concentration-QTc relationship. The observed ΔQTcF values from the active group will be adjusted by the estimated time effect from the concentration-QTc model (i.e., the estimated diurnal effect under the placebo treatment). The individually estimated placebo-adjusted $\Delta QTcF_{i,j}$ ($\Delta\Delta QTcF_{i,j}$) equals the individual $\Delta QTcF_{i,j}$ for subject i administered with OM at time point j minus the estimation of time effect at time point j. Additional plots will be used to validate the model assumptions. Exploratory analyses (via graphical displays and/or model fitting) will also include accounting for a delayed effect (hysteresis; Section 6.3.1) and the justification for the choice of pharmacodynamic model (linear versus nonlinear; Section 6.3.2).

The SAS code for the concentration-QTc analysis 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;
ESTIMATE 'Pred Mean Diff for T1' TRT 1 CONC &GeoMeanCmax_1 / CL ALPHA=0.1;
RUN;
```

Where PKQTc =PK/QTc analysis set, SUBJID=subject identifier, TRT=treatment (active=1 or placebo=0), TIME=nominal time point, CONC=OM plasma concentration, CBASE=centered baseline QTcF, T1 = therapeutic oral dose of OM, GeoMeanCmax_1=geometric mean C_{max} for T1, and DQTC=ΔQTcF.

6.3.1 Investigation of Hysteresis

Hysteresis will be assessed based on joint graphical displays of the LS mean difference between ΔQTcF under OM and under placebo (ΔΔQTcF) for each postdose time point and the mean concentrations of OM at the same time points. In addition, hysteresis plots will be given for LS mean ΔΔQTcF in the by-time point analysis and the mean concentrations. If a QT effect above 10 ms (i.e., LS mean ΔΔQTcF > 10 ms) cannot be excluded from the by-time point analysis and the mean peak ΔQTcF effect is observed at the same time point in the by-time point analysis, and if a delay between peak ΔΔQTcF and peak plasma concentration exceeds 1 hour in a consistent way, other concentration-QTc models, such as a model with an effect compartment, may be explored. With the provision stated above, hysteresis will be assumed if the curve of hysteresis plot shows a counterclockwise loop.

6.3.2 Appropriateness of a Linear Model

To assess the appropriateness of a linear model, normal quantile-quantile (Q-Q) plots for the standardized residuals and random effects; scatter plots of standardized residuals versus concentration, fitted values, and centered baseline QTcF; and box plots of standardized residuals versus nominal time, and active treatment will be produced. The scatter plots of standardized residuals versus concentration and versus centered baseline QTcF by LOESS fitting (i.e., locally weighted scatter plot smoothing as described by Cleveland[4]) will also be produced with optimal smoothing parameters selected by the Akaike information criterion with a correction.[5] A scatter plot of observed concentration and ΔQTcF with 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 will be fitted, in particular, an E_{max} model. The concentration-QTc analysis will then be repeated for the model found to best accommodate the nonlinearity detected.

6.4 Determination of Sample Size

With approximately 60 enrolled subjects, it is estimated that approximately 48 evaluable subjects will have data from all 3 periods in Part B.

Based on the calculation of the sample size for a TQT study[6], assuming a 1-sided 5% significance level and a within-subject SD of 7 ms for ΔQTcF, and a true mean difference of 3 ms in ΔQTcF between OM and placebo, a sample size of 48 evaluable subjects would be expected to provide 99.5% power to demonstrate that the upper bounds of all the 2-sided 90% CIs on ΔΔQTcF will fall below 10 ms for up to 8 time points. With the within-subject SD of 8 ms and other similar assumptions, the power will decrease to 96.5%.

Determination of sample size for assay sensitivity

Assuming a 1-sided 5% significance level and a within-subject SD of 7 ms for ΔQTcF, a sample size of 48 evaluable subjects will provide a power of 99.9% to exclude a mean difference of 5 ms in ΔQTcF between moxifloxacin and placebo groups from the lower bound of the 2-sided 90% CI on ΔΔQTcF at at least 1 of the 3 prespecified time points. With the within-subject SD of 8 ms and other similar assumptions, the power will decrease to 99%.

7 References

1. Hochberg Y. A sharper Bonferroni procedure for multiple tests of significance. *Biometrika*. 1988;75:800-02.
2. 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.
3. 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.
4. Cleveland WS. Robust locally weighted regression and smoothing scatterplots. *J Am Stat Assoc*. 1979;74(368):829-836.

5. 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.
6. Zhang J and Machado SG. Statistical issues including design and sample size calculation in thorough QT/QTc studies. *J Biopharm Stat*. 2008;18(3):451-67.

8 Tables, Figures, and Listings

8.1 Tables

Number	Title	Comments
14.1.1	Baseline values of ECG parameters with descriptive statistics	Number of subjects (n), mean, SD, 90% CI, median, minimum, and maximum from descriptive analysis will be given by treatment for each ECG parameter (Section 6.1).
14.1.2.1 – 14.1.2.4	Observed values of QTcF, HR, PR, and QRS with descriptive statistics	n, mean, SD, SE, 90% CI, median, minimum, and maximum from descriptive statistics will be given by treatment and postdose time point (Section 6.1).
14.1.3.1-14.1.3.4	Change-from-baseline QTcF, HR, PR, and QRS (ΔQTcF, 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 6.1).
14.1.4.1-14.1.4.4	Placebo-corrected change-from-baseline QTcF, HR, PR, and QRS (ΔΔQTcF, 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 6.1).
14.1.5	Placebo-corrected change-from-baseline QTcF (ΔΔQTcF) for assay sensitivity test	LS mean, Lower bound of 2-sided 90% CI, *P* value, and adjusted alpha will be given at each of the 3 prespecified time points (Section 6.1.2).
14.1.6	QTcF outliers per absolute category	Number (%) of subjects and time points with QTcF > 450 and ≤ 480 ms, > 480 and ≤ 500 ms, or > 500 ms by treatment (Section 6.2).
14.1.7	QTcF outliers per change-from-baseline category	Number (%) of subjects and time points with ΔQTcF > 30 and ≤ 60 ms, or > 60 ms by treatment (Section 6.2).

Number	Title	Comments
14.1.8	Categorical analyses for HR, PR, and QRS	Number (%) of subjects and time points with ΔPR > 25% and PR > 200 ms at post-baseline; ΔQRS > 25% and QRS > 120 ms at post-baseline; HR decrease from baseline > 25% and HR < 50 bpm at post-baseline; and HR increase from baseline > 25% and HR > 100 bpm at post-baseline (Section 6.2).
14.1.9	T-wave morphology and U-wave presence across treatments: treatment-emergent changes	Number (%) of subjects and time points falling into each of the T-wave categories: Normal (+), Flat, Notched (+), Biphasic, Normal (-), Notched (-) as defined in Section 6.2.
14.1.10	Concentration-QTc analysis of OM and associated ΔQTcF prolongation	Fixed-effect estimations and corresponding *P* values will be given (Section 6.3).
14.1.11	Predicted ΔΔQTcF interval at geometric mean peak OM concentration	Section 6.3.

8.2 Figures

Number	Title	Comments
14.2.1	Observed QTcF across time points	Mean and 90% CI from descriptive analysis will be given by treatment (Section 6.1).
14.2.2.1-14.2.2.4	Change-from-baseline QTcF, HR, PR, and QRS (ΔQTcF, ΔHR, ΔPR, and ΔQRS) across time point	LS mean and 90% CI from the statistical modeling will be shown by treatment (Section 6.1).
14.2.3.1-14.2.3.4	Placebo-corrected change-from-baseline QTcF, HR, PR, and QRS (ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS) across time point	LS mean and 90% CI from the statistical modeling will be shown by treatment (Section 6.1).
14.2.4.1	Scatter plot of QTcF versus RR by treatment	Scatter plots of QTcF and RR intervals by treatment with regression lines will be given (Section 5.4).
14.2.4.2	QTcF-RR quantile plot by treatment	QTcF-RR quantile plots (with quantiles) with linear mixed-effects line and 90% CI will be given (Section 5.4).
14.2.5	Mean OM plasma concentrations over time	Section 6.3.

Number	Title	Comments
14.2.6	Joint plot of OM plasma concentrations and ΔΔQTcF over time	Section 6.3.1.
14.2.7	Hysteresis plot of OM plasma concentration and ΔΔQTcF connected in temporal order by dose	Section 6.3.1.
14.2.8	Scatter plot of observed OM plasma concentrations and ΔQTcF	Scatter plot of ΔQTcF versus concentration with LOESS line and 90% CI and simple regression line (Section 6.3.2).
14.2.9	Scatter plot of observed OM plasma concentrations and estimated placebo-adjusted ΔQTcF	Scatter plot of placebo-adjusted ΔQTcF versus concentration with linear mixed-effects regression line and 90% CI (Section 6.3).
14.2.10	Model-predicted ΔΔQTcF (mean and 90% CI) and estimated placebo-adjusted ΔQTcF (mean and 90% CI) across deciles of OM plasma concentrations	Section 6.3.
14.2.11	Predicted ΔΔQTcF interval at geometric mean peak OM concentrations	Section 6.3.
14.2.12	Scatter plot of standardized residuals versus fitted values	Section 6.3.2.
14.2.13	Scatter plot of standardized residuals versus concentrations with LOESS	Section 6.3.2.
14.2.14	Scatter plot of standardized residuals versus centered baseline QTcF with LOESS	Section 6.3.2.
14.2.15	Box plot of standardized residuals versus nominal time	Section 6.3.2.
14.2.16	Box plot of standardized residuals versus treatment	Section 6.3.2.
14.2.17	Normal Q-Q plot of standardized residuals	Section 6.3.2.
14.2.18	Normal Q-Q plots of the estimated random effects	Section 6.3.2.

8.3 Listings

Number	Title	Comments
16.2.1.1-16.2.1.4	QTcF, HR, PR, and QRS intervals – absolute and change-from-baseline values	Section 6.1.
16.2.2	T-wave morphology and U-wave presence	Section 6.2.
16.2.3	ΔQTcF and time-matched OM concentrations for each subject	Data for concentration-QTc analysis (Section 6.3).

9 Approvals

ERT

[redacted]

[redacted]
MD PhD
approved
16 April 2020 14:54 +02:00
cosign

[redacted] MD, PhD
Chief Scientific Officer, Cardiac Safety

Date

[redacted]

[redacted]
Senior Biostatistician
approval
16 April 2020 09:03 -04:00
cosign

[redacted], PhD
Principal Biostatistician

Date

[redacted]

[redacted]
Project Manager
Reviewed and Approved
16 April 2020 09:54 -04:00
cosign

[redacted]
Project Manager

Date

Amgen Inc.

[redacted]

4/15/2020

[redacted]
Executive Director Biostatistics

Date

Appendix A: Expert Precision QT Analysis

Expert 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.