16.1.9. Documentation of Statistical Methods

16.1.9.1. Statistical Analysis Plan

Statistical Analysis Plan

A Phase I, Multicenter, Randomized, Double-blind, Double-dummy, Placebo- and Positive-Controlled Study to Investigate the Effects of CBP-307 on the QT_C Interval in Healthy Subjects

SAP Status: Final
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LIST OF ABBREVIATIONS

Abbreviations pertain to the statistical analysis plan (SAP) only (not the tables, figures, and listings [TFLs]).

ADaM	Analysis Data Model
AE	adverse event
AUC_{0-24}	area under the concentration-time curve from time zero to 24 hours postdose
AUC_{inf}	area under the concentration-time curve from time zero extrapolated to infinity
BLQ	below the limit of quantification
CDISC	Clinical Data Interchange Standards Consortium
C_{max}	maximum observed concentration
COVID-19	coronavirus disease 2019
CSR	clinical study report
DMP	data management plan
ECG	electrocardiogram
eCRF	electronic case report form
EOS	end of study
HR	heart rate
ICF	informed consent form
ICH	International Council for/Conference on Harmonisation
MedDRA	Medical Dictionary for Regulatory Activities
PK	pharmacokinetic(s)
PR	pulse rate
QTc	heart-rate corrected QT interval
QTcB	QT interval corrected for heart rate using Bazett's formula
QTcF	QT interval corrected for heart rate using Fridericia's formula
SAP	statistical analysis plan
SD	standard deviation
SDV	source document verification
TEAE	treatment-emergent adverse event
TFL	table, figure, and listing
t_{max}	time of the maximum observed concentration
WHODrug	World Health Organization Drug Dictionary
Δ	change-from-baseline
$\Delta\Delta$	placebo-corrected or placebo-adjusted change-from-baseline

1. INTRODUCTION

This SAP has been developed after review of the clinical study protocol Amendment 4 (Protocol Version 5.0 dated 12 January 2022) and electronic case report form (eCRF).

This SAP describes the planned analysis of the pharmacokinetic (PK), safety, and tolerability data from this study. A detailed description of the planned TFLs to be presented in the clinical study report (CSR) is provided in the accompanying TFL shells document.

In general, the analyses are based on information from the protocol, unless they have been modified by agreement with Connect Biopharma Australia Pty Ltd. A limited amount of information about this study (eg, objectives, study design) is given to help the reader's interpretation.

This SAP must be finalized prior to any unblinding of study data. Additionally, the SAP and TFL shells should be finalized prior to any programming activities commencing.

This SAP supersedes any statistical considerations identified in the protocol; where considerations are substantially different, they will be so identified. If additional analyses are required to supplement the planned analyses described in this SAP, they may be performed and will be identified accordingly in the CSR. Any substantial deviations from this SAP will be agreed with Connect Biopharma Australia Pty Ltd. and identified in the CSR.

This SAP is written with consideration of the recommendations outlined in the International Conference on Harmonisation (ICH) E3 guideline *Structure and Content of Clinical Study Reports*, ICH E8 guideline *General Considerations for Clinical Trials*, ICH E9 guideline *Statistical Principles for Clinical Trials*.[1,2,3]

The document history is presented in Appendix 1.

2. STUDY OBJECTIVES

The primary objective of the study is:

• To evaluate the effects of therapeutic and supratherapeutic CBP-307 plasma concentrations on the heart-rate corrected QT interval (QTc) in healthy subjects.

The secondary objectives of the study are:

• To demonstrate assay sensitivity of the study to detect a small QT effect using moxifloxacin as a positive control.

• To evaluate the safety and tolerability of therapeutic and supratherapeutic multiple oral doses of CBP-307 in healthy subjects.

• To assess the pharmacokinetics (PK) of CBP-307 following administration of therapeutic and supratherapeutic multiple oral doses in healthy subjects.

• To evaluate the effects of therapeutic and supratherapeutic multiple oral doses of CBP-307 on heart rate (HR), pulse rate (PR) and QRS intervals, and T-wave morphology.

Note: This SAP only focuses on the PK, safety and tolerability objectives. All QTc related objectives will be covered in a separate report provided by ERT Inc.

3. STUDY ENDPOINTS

Electrocardiogram Endpoints:

The primary endpoint is the change-from-baseline QTcF (ΔQTcF).

The secondary endpoints are:

• Change-from-baseline HR, PR, QRS (ΔHR, ΔPR, and ΔQRS);

• Placebo-corrected change-from-baseline HR, QTcF, PR, and QRS (ΔΔHR, ΔΔQTcF, ΔΔPR, and ΔΔQRS);

• Categorical outliers for QTcF, HR, PR, and QRS;

• Frequency of treatment-emergent changes of T- wave morphology and U-wave presence.

Pharmacokinetic Endpoints:

• area under the concentration-time curve from time zero extrapolated to infinity (AUC_{inf})

• area under the concentration-time curve from time zero to 24 hours postdose (AUC_{0-24})

• maximum observed concentration (C_{max})

• time of the maximum observed concentration (t_{max})

Other PK parameters may also be reported.

Safety Endpoints:

The safety outcome measures for this study are as follows:

• incidence and severity of AEs

• incidence of laboratory abnormalities, based on hematology, clinical chemistry, and

urinalysis test results

• 12-lead ECG parameters

• vital signs measurements.

Note: This SAP only focuses on the PK, safety and tolerability endpoints. All QTc related endpoints will be covered in a separate report provided by ERT Inc.

4. STUDY DESIGN

This will be a Phase I, randomized, double-blind, double-dummy, placebo-controlled, positive-controlled, multi-site, 3-arm study to investigate the effects of therapeutic and supratherapeutic oral doses of CBP-307 on the QTc interval in healthy male and female subjects.

Potential subjects will be asked to read and sign an informed consent form (ICF). After informed consent is obtained, screening procedures and tests to establish subject eligibility to enter the study will be performed within 28 days before Day 1. After informed consent has been obtained but prior to the initiation of study treatment administration, only SAEs will be reported. After placebo administration to all subjects on Day -1, all AEs, whether volunteered, elicited, or noted upon physical examination, will be recorded throughout the study (ie, from Day -1 until the end of the study).

In this study, approximately 68 healthy subjects (at least 30% for each sex) will be randomized into 2 groups (Group 1 and 2) with 34 subjects in each. Group 2 consists of 2 sub-groups (Group 2A and 2B) and each sub-group will be randomized with 17 subjects. Randomized subjects will receive the assigned study drug as a single dose in the morning on Days 1, 2, 3 to 6, 7 to 15, and on Day 16 (placebo will be administered to all subjects on Day -1).

The following treatments will be administered:

• Starting doses of CBP-307 for titration (0.05 mg on Day 1 followed by an up-titrated dose of 0.1 mg on Day 2)

• A therapeutic dose of CBP-307 (0.2 mg, Days 3 to 6)

• A supratherapeutic dose of CBP-307 (0.5 mg, Days 7 to 15)

• Placebo (matched to moxifloxacin and CBP-307)

• Moxifloxacin (400 mg)

Moxifloxacin will be used as a positive control to determine the assay sensitivity of this study with an expected peak QT effect (placebo-corrected change-from-baseline QTcF; ΔΔQTcF) of 10 to 15 msec.

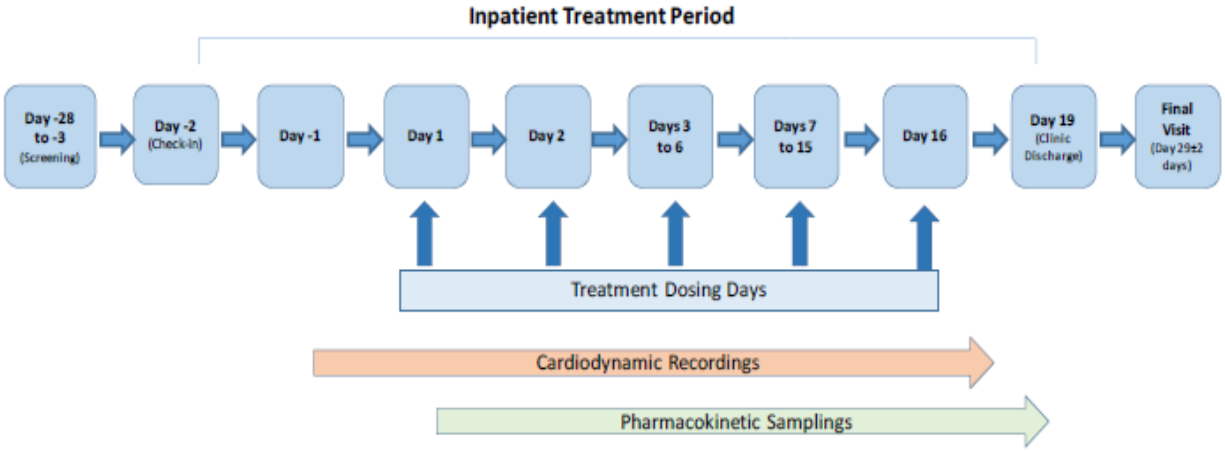
A summary of dosing timelines per Group is shown below:

Study Treatment Name	Treatment Group	CBP-307	CBP-307 Matched Placebo	Moxifloxacin (Avelox)	Moxifloxacin Matched Placebo
Dosage Formulation		Capsule	Capsule	Tablet	Tablet
Unit Dose Strength(s)/Level(s)		0.05 mg 0.1 mg	Not applicable	400 mg	Not applicable
Dosage Frequency					
Day -1	Group 1		1 capsule		
	Group 2A		1 capsule		
	Group 2B		1 capsule		
Day 1	Group 1	1 × 0.05 mg			1 tablet
	Group 2A		1 capsule	1 × 400 mg	
	Group 2B		1 capsule		1 tablet
Day 2	Group 1	1 × 0.1 mg			
	Group 2A		1 capsule		
	Group 2B		1 capsule		
Days 3 to 6	Group 1	2 × 0.1 mg			
	Group 2A		2 capsules		
	Group 2B		2 capsules		
Days 7 to 15	Group 1	5 × 0.1 mg			
	Group 2A		5 capsules		
	Group 2B		5 capsules		
Day 16	Group 1		1 capsule		1 tablet
	Group 2A		1 capsule		1 tablet
	Group 2B		1 capsule	1 × 400 mg	

A schematic of the study design is presented in

Figure 1.

Figure 1: Study Design



Potential subjects will be screened to assess their eligibility to enter the study within 27 days prior to the first dose administration. Subjects will be admitted into the study site on Day -2 (or on Day -4 depending on the clinical site's requirement for Coronavirus Disease 2019 [COVID-19] testing) and will remain at the study site until discharge on Day 19. Treatment administration will occur on Days 1, 2, 3 to 6, 7 to 15, and 16 under fasting conditions (all subjects will receive placebo on Day -1). Continuous cardiodynamic ECG monitoring and recording will begin at least 25 hours prior to the administration of study treatments on Day 1 and continue through 24 hours after the administration of study treatments on Days 1, 6, 15, and 16 and at corresponding timepoints on the day before dosing, ie, Day -1. Blood samples for PK analysis will be collected predose and at each postdose cardiodynamic ECG timepoint. Additional PK samples will be collected at 72 and 96 hours following dosing on Day 15. Subjects will return to the study site for a follow-up visit on Day 29±2 days.

Baseline for the primary analysis will be determined from predose timepoints collected for 25 hours on Day -1 (prior to administration of study treatments on Day 1). Continuous cardiodynamic monitoring (Holter) will be performed for 25 hours on Days -1, 1, 6, 15, and 16. The recording will be started 1 hour before dosing and at the corresponding clock time on Day -1. Replicate 12-lead ECGs will be extracted from the continuous recording prior to dosing (-30 minutes) and 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours postdose on each day. Pharmacokinetic blood samples will be collected at the following timepoints: prior to dosing and 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours after dosing on each PK sampling day (Days 1, 6, 15, and 16; sample collection windows are as follows: ±2 minutes for 0.5 hours postdose, ±5 minutes for 1 to 4 hours postdose, ±10 minutes for 6 to 8 hours postdose, and ±30 minutes for 12 to 24 hours postdose). Additional PK samples will be collected at 72 and 96 hours following dosing on Day 15 (sample collection window: ±30 minutes). Subjects will be inactive and in a supine position for at least 10 minutes prior to and 5 minutes after each cardiodynamic ECG extraction timepoint, which will end immediately prior to the PK sampling. At each timepoint, up to 10 ECG replicates will be extracted from a 5-minute time window (the last 5 minutes of the 15-minute period when the subject is maintained in a supine, quiet position). The ECG effects of CBP-307 will be tested on data from Day -1 (baseline for both groups), Day 6 (therapeutic concentrations versus placebo), and Day 15

(supratherapeutic concentrations versus placebo). Assay sensitivity with moxifloxacin will be evaluated using ECG data collected on Days -1, 1, 15, and 16.

The central ECG laboratory (eResearch Technology Inc., Philadelphia, PA) will be blinded to treatment. The continuous 12-lead digital ECG data will be exported from the system and sent to the central ECG laboratory for review. Resting ECGs to be used in the analyses will be selected from predetermined timepoints specified above and will be read by the central ECG laboratory.

Safety assessments including AEs, vital signs, clinical laboratory tests, safety 12-lead ECGs, and physical examination findings will be performed at screening and at specific timepoints during the study, and/or at the follow-up visit. In addition, subjects will be monitored via cardiac telemetry at specific timepoints during the treatment period.

The total duration of study participation for each subject (from screening through follow-up visit) is anticipated to be approximately 59 days.

The start of the study is defined as the date the first subject signs an ICF. The point of enrollment occurs at the time of subject number allocation. The end of the study is defined as the date of the last subject's last assessment (scheduled or unscheduled).

5. SAMPLE SIZE JUSTIFICATION

Approximately 64 healthy subjects (at least 30% for each sex) will be randomized into 2 groups (Group 1 and 2) with 32 subjects in each. Group 2 consists of 2 sub-groups (Group 2A and 2B) and each sub-group will be randomized with 16 subjects.

Sample Size for Primary Analysis:

A sample size of 28 evaluable subjects per treatment group will provide more than 94.4% power to exclude that CBP-307 causes more than 10-msec QTc effect at clinically relevant plasma levels, as shown by the upper bound of the 2-sided 90% confidence interval (CI) of the model-predicted QT effect (ΔΔQTcF) at the observed geometric mean Cmax of CBP-307 in the study. This power is estimated approximately using a 2-sample t-test. The calculation assumes a 1-sided 5% significance level, an underlying effect of CBP-307 of 3 msec and a standard deviation (SD) of the ΔQTcF of 8 msec for both CBP-307 and placebo treatment groups. Note that this calculation is conservative, since it does not take into account any gain in precision due to the use of all data of each subject with the help of a linear mixed-effects model. The concentration-QTc analysis method is supported by Darpo et al 2015[4] and Ferber et al, 2015,[5] and consistent with the experiences from 25 recent TQT studies.

Sample Size Considerations for Assay Sensitivity:

To demonstrate assay sensitivity with concentration-QTc analysis, it has to be shown that the ΔΔQTcF of a single dose of 400 mg moxifloxacin exceeds 5 msec (ie, the lower bound of the 2-sided 90% CI of the predicted QTc effect [ΔΔQTcF] should exceed 5 msec). 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 exposure-response

analysis was 1.24 msec. The within-subject SD of ΔQTcF in the referred study was 5.4 msec based on the by-timepoint analysis. If the effect of moxifloxacin is assumed to be 10 msec, the SE of 1.24 msec 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 0.64 required to guarantee a power of at least 95% in a paired t-test situation with a sample size of 28 evaluable subjects. In other words, based on this calculation, a power of at least 95% will be obtained as long as the variability of the ΔQTcF, as measured by its within-subject SD from the by-timepoint analysis, does not exceed 6.9 msec (ie, 128% [= 0.82/0.64] of the 5.4 msec 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.[6]

6. STUDY TREATMENTS

The study treatment names and ordering to be used in the TFLs are presented in Table 1.

Table 1: Presentation of Study Treatments in TFLs

Study Treatment	Order in TFLs
Placebo	1
400 mg Moxifloxacin	2
0.05 mg CBP-307	3
0.1 mg CBP-307	4
0.2 mg CBP-307	5
0.5 mg CBP-307	6

All TFLs will be based on actual treatments (eg, if subject was assigned to receive placebo but was wrongfully dosed with active treatment they would be summarized and listed under active treatment).

The study treatment sequence names, abbreviations, and ordering to be used in the TFLs are presented in Table 2.

Table 2: Presentation of Study Treatment Sequences in TFLs

Study Treatment Sequence	Abbreviation	Order in TFLs
1 capsule of CBP-307 matched placebo on Day -1 -> 1 × 0.05 mg CBP-307 and 1 tablet of Moxifloxacin matched placebo on Day 1 -> 1 × 0.1 mg CBP-307 on Day 2 -> 2 × 0.1 mg CBP-307 on Days 3 to 6 -> 5 × 0.1 mg CBP-307 on Days 7 to 15 -> 1 capsule of CBP-307 matched placebo and 1 tablet of Moxifloxacin matched placebo on Day 16	Group 1	1
1 capsule of CBP-307 matched placebo on Day -1 -> 1 capsule of CBP-307 matched Placebo and 1 × 400 mg Moxifloxacin tablet on Day 1 -> 1 capsule of CBP-307 matched Placebo on Day 2 -> 2 capsules of CBP-307 matched Placebo Days 3 to 6 -> 5 capsules of CBP-307 matched Placebo on Days 7 to 15 -> 1 capsule of CBP-307 matched placebo and 1 tablet of Moxifloxacin matched placebo on Day 16	Group 2A	2
1 capsule of CBP-307 matched placebo on Day -1 -> 1 capsule of CBP-307 matched Placebo and 1 tablet of Moxifloxacin matched placebo on Day 1 -> 1 capsule of CBP-307 matched Placebo on Day 2 -> 2 capsules of CBP-307 matched Placebo Days 3 to 6 -> 5 capsules of CBP-307 matched Placebo on Days 7 to 15 -> 1 capsule of CBP-307 matched placebo and 1 × 400 mg Moxifloxacin tablet on Day 16	Group 2B	3

The summaries will be based on planned treatment sequences and listings will be based on actual treatment and sequences.

7. DEFINITIONS OF POPULATIONS

Any protocol deviations, including those due to coronavirus disease 2019 (COVID-19) and related restrictions (see Section 8.1.1), will be considered prior to database lock for their importance and taken into consideration when assigning subjects to populations.

7.1. All Subjects Population

The all subjects population will include all subjects who signed the informed consent form and had any study assessment recorded in the database per the protocol.

7.2. Safety Population

The safety population will include all subjects who received at least 1 dose of investigational product drug starting from Day -1 (therapeutic and supratherapeutic doses of CBP-307, moxifloxacin, or placebo).

7.3. Pharmacokinetic Population

The PK population will include all subjects who received at least 1 dose of investigational product and have evaluable PK data of any of the analytes (CBP-307 and moxifloxacin). 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 median time to maximum concentration.

7.4. Cardiodynamic Population

The cardiodynamic population will be specified in the report from ERT Inc. and not included in this SAP.

8. STATISTICAL METHODOLOGY

8.1. General

Listings will be provided for all data captured in the database, including medical history. Listings will include all subjects assigned to the all subjects population and include data up to the point of study completion or discontinuation. Subjects are generally considered to have completed the study if they complete the scheduled follow-up visit (rather than early termination visit). Any subject who discontinues the study will be identified accordingly in the listings. Summaries and statistical analyses will include the subjects assigned to the relevant population based on data type.

Data analysis will be performed using the SAS® statistical software package Version 9.4 (or higher if a new version is issued during the study).

Analysis Data Model (ADaM) datasets will be prepared using Clinical Data Interchange Standards Consortium (CDISC) ADaM Version 2.1 (or higher if a new version is issued during the study) and CDISC ADaM Implementation Guide Version 1.1 (or higher if a new version is issued during the study). Pinnacle 21 Community Validator Version 3.1.0 (or higher if a new version is issued during the study) will be utilized to ensure compliance with CDISC standards.

Where reference is made to ‘valid’ data, this refers to non-missing data which meet the predetermined criteria (eg, are not flagged for exclusion).

Where reference is made to ‘all calculations’, this includes, but is not limited to, summary statistics, statistical analyses, baseline derivation and changes from baseline.

All figures will be produced on linear-linear or discrete-linear scales, as applicable, unless specifically stated otherwise.

8.1.1. Handling of Data Quality Issues Due to Coronavirus Disease 2019 and Related Restrictions

Due to COVID-19 and related restrictions, there is a high risk for impact to data integrity, with the recognized potential for:

- Missed visits, caused by, for example,:
  - Subject unable to travel to site due to restrictions, the need to quarantine, or COVID-19 infection
  - Subject unwilling to go to site due to fear of COVID-19 infection
  - Site postponing subject's visit due to investigator not being available (eg, if they have been dispatched to hospital handling COVID-19 infections)
- Site unable to replenish supply of investigational product
- Incomplete data entry by sites due to limited resources to support study or no access to source documents or to eCRF
- Outstanding source document verification (SDV) due to sponsor or country restrictions on remote SDV, or no or limited access to site(s) for on-site visits
- Unanswered queries

At the time of the reporting of the study results, all protocol deviations due to COVID-19 or related restriction will be assessed for their severity and impact on the analyses. If needed, appropriate statistical methods will be applied as a mitigating action (eg, data might be categorized into 2 analysis groups, with and without COVID-19 and related restrictions impact); however, this will exclude any imputations of the missing values. Any mitigating actions will be agreed with Connect Biopharma Australia Pty Ltd in advance and identified in the CSR.

8.1.2. Calculation of the Summary Statistics

For continuous data the following rules will be applied:

- Missing values will not be imputed, unless specifically stated otherwise.
- Unrounded data will be used in the calculation of summary statistics.
- If the number of subjects with valid observations (n) <3, summary statistics will not be calculated, with the exception of n, minimum, and maximum.
- In general, as early termination data are not associated with any scheduled timepoint, they will be excluded from all calculations of summary statistics and statistical analyses. Exceptions may be made where justified.

For categorical data the following rules will be applied:

- For ordered categorical data (eg, adverse event [AE] severity), all categories between the possible minimum and maximum categories will be included, even if n = 0 for a given category.

- For non-ordered categorical data (eg, race), only those categories for which there is at least 1 subject represented will be included; unless specifically stated otherwise.
- Missing values will not be imputed, unless specifically stated otherwise. A 'missing' category will be included for any parameter for which information is missing. This will ensure that the population size totals are consistent across different parameters.

8.1.3. Triplicate Readings

For 12-lead electrocardiogram (ECG) data only, where triplicate readings are taken, the mean of triplicate readings will replace the separate individual triplicate readings in all calculations.

In case of incomplete triplicate readings (eg, only 2 out of 3 readings were recorded), the mean and/or medians will be calculated, as appropriate, based on the number of readings available.

8.1.4. Repeat and Unscheduled Readings

For vital signs and 12-lead ECG data only, any predose value recorded in addition to the original value or a postdose value recorded within 15 minutes of the original value will be defined as a repeat value; any postdose value recorded more than 15 minutes after the original value will be defined as an unscheduled value. For all other data types (eg, laboratory parameters), any value recorded in addition to the original value will be defined as an unscheduled value.

As unscheduled values are not associated with any scheduled timepoint, they will be excluded from all calculations unless specifically stated otherwise.

8.1.5. Definitions of Baseline and Change from Baseline

The baseline will be defined as the last value recorded prior to dosing. If the date/time of the value is incomplete or missing, it will be excluded from the baseline calculation, unless the incomplete date/time indicates the value was recorded prior to dosing.

Individual changes from baseline will be calculated by subtracting the individual subject's baseline value from the value at the postdose timepoint.

The summary statistics for change from baseline will be derived from individual subjects' values (eg, mean change from baseline will be the mean of the individual changes from baseline for all subjects, rather than difference between the mean value at the postdose timepoint and mean value at baseline).

See Section 8.1.4 for more detail on handling repeat and unscheduled readings in the calculations. See Section 8.1.3 for more detail on handling of triplicate readings in the calculations.

8.2. Subject Disposition and Population Assignment

Subject disposition and population assignment will be listed.

A summary table by group treatment sequence will be provided, based on the safety population.

Screen failure data summary table will be provided separately, based on the all subjects population.

8.3. Screening Demographics and Baseline Characteristics

The screening demographics and baseline characteristics including age, sex, race, ethnicity, height, body weight, and body mass index will be listed.

A summary table by group treatment sequence will be provided, based on the safety population.

8.4. Prior and Concomitant Medication

Prior medication will be defined as medication that ends prior to dosing. Concomitant medication will be defined as medication that starts during or after dosing or starts but does not end prior to dosing.

Prior and concomitant medications will be coded using the World Health Organization Drug Dictionary (WHODrug) Global, Format B3, Version March 2021 (or later if a new version is issued during the study; see the data management plan [DMP] for more details). Prior and concomitant medications will be listed.

8.5. Pharmacokinetic Assessments

8.5.1. Pharmacokinetic Analysis

The following PK parameters will be determined where possible from the plasma concentrations of CBP-307 and moxifloxacin using noncompartmental methods in validated software program Phoenix WinNonlin (Certara, Version 8.1 or higher):

Moxifloxacin (Days 1 and 16)		
Parameter	**Units[a]**	**Definition**
C_{max}	ng/mL	maximum observed concentration
t_{max}	h	time of the maximum observed concentration
$AUC_{0\text{-}tlast}$	h*ng/mL	area under the concentration-time curve from time 0 to the time of the last quantifiable concentration (t_{last})[b]
$AUC_{0\text{-}24}$	h	area under the concentration-time curve over the time interval from 0 to 24 hours post dose
CBP-307 (Days 1, 6 and 15)		

AUC_{inf}	h*ng/mL	area under the concentration-time curve from time 0 extrapolated to infinity[c] (Day 15 only)
$AUC_{0\text{-}tlast}$	h*ng/mL	area under the concentration-time curve from time 0 to the time of the last quantifiable concentration (t_{last})[b]
$\%AUC_{extrap}$	%	percentage of area under the concentration-time curve due to extrapolation from the last quantifiable concentration to infinity (Day 15 only)
$AUC_{0\text{-}24}$	h	area under the concentration-time curve over the time interval from 0 to 24 hours post dose
C_{max}	ng/mL	maximum observed concentration
t_{max}	h	time of the maximum observed concentration
t_{last}	h	time of the last quantifiable concentration
$t_{1/2}$	h	apparent terminal elimination half-life (Day 15 only)
CL/F	L/h	apparent total clearance (Day 15 only)
V_z/F	L	apparent volume of distribution during the terminal phase (Day 15 only)

[a] Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study.
[b] The AUC will be calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations (linear up/log down rule).
[c] Based on the last observed quantifiable concentration

Additional PK parameters may be determined where appropriate.

Pharmacokinetic analysis will be carried out where possible using actual dose administered (mg) and actual postdose blood sampling times. If an actual time is missing, the sample concentration result will be treated as missing unless there is scientific justification to include the result using the nominal time.

The parameters C_{max}, t_{last}, and t_{max} will be obtained directly from the concentration-time profiles. If C_{max} occurs at more than 1 timepoint, t_{max} will be assigned to the first occurrence of C_{max}.

8.5.1.1. Criteria for the Calculation of Apparent Terminal Elimination Rate Constant and Half-life

The start of the terminal elimination phase for each subject will be defined by visual inspection and generally will be the first point at which there is no systematic deviation from the log-linear decline in concentrations.

The apparent terminal elimination rate constant (λ_z) will only be calculated when a reliable estimate can be obtained using at least 3 data points, preferably not including C_{max}, and the adjusted coefficient for determination of exponential fit (R^2-adj) of the regression line is ≥0.7. Parameters requiring λ_z for their calculation (eg, AUC_{inf}, $t_{1/2}$, CL/F, and V_z/F for Profile Day 15) will only be calculated if the R^2-adj value of the regression line is ≥0.7.

The following regression-related diagnostic PK parameters will be determined, when possible:

Parameter	Units	Definition
λ_z	1/h	apparent terminal elimination rate constant
λ_z Upper	h	end of exponential fit
λ_z Lower	h	start of exponential fit
λ_z N	NA	number of data points included in the log-linear regression
λ_z Span Ratio	NA	time period over which λ_z was determined as a ratio of $t_{1/2}$
R^2-adj	NA	adjusted coefficient for determination of exponential fit

Where possible, the span of time used in the determination of λ_z (ie, the difference between λ_z Upper and λ_z Lower) should be ≥2 half-lives. If the λ_z Span Ratio is <2, the robustness of the $t_{1/2}$ values will be discussed in the clinical study report (CSR).

8.5.1.2. Criteria for Calculation and Reporting of Area Under the Concentration-time Curve

The minimum requirement for the calculation of AUC will be the inclusion of at least 3 consecutive concentrations above the lower limit of quantification. If there are only 3 consecutive concentrations, at least 1 should follow C_{max}.

If the extrapolated area is >20%, AUC_{inf} (and derived parameters) may be excluded from the summary statistics at the discretion of the sponsor or pharmacokineticist.

8.5.1.3. Criteria for Handling Concentration Below the Limit of Quantification or Missing Concentrations for Pharmacokinetic Analysis

Plasma concentrations below the limit of quantification (BLQ) will be assigned a value of 0 before the first measurable concentration and thereafter BLQ concentrations will be treated as missing. The following rules apply to the specific situations defined below:

- If an entire concentration-time profile is BLQ, it will be excluded from PK analysis.
- Where 2 or more consecutive concentrations are BLQ at the end of a profile, the profile will be deemed to have terminated and any further quantifiable concentrations will be set to missing for the calculation of the PK parameters, unless they are considered to be a true characteristic of the profile of the drug.
- If a predose plasma concentration is missing, it will be set to 0 by default within Phoenix WinNonlin for the first dosing day (Study Profile Day 1).
- For multiple dose part of the study (Study Profile Day 15), if the CBP-307 concentration at 24 hours postdose is missing, this may be substituted with the predose.

8.5.1.4. Treatment of Outliers in Pharmacokinetic Analysis

If a value is considered to be anomalous due to being inconsistent with the expected PK profile, it may be appropriate to exclude the value from the PK analysis. However, the exclusion of any data must have strong justification and will be documented in the CSR.

Any quantifiable predose concentration value on the first dosing day (Study Profile Day 1) will be considered anomalous and set to missing for the PK analysis. This will be set to 0 by default in Phoenix WinNonlin.

8.5.2. Presentation of Pharmacokinetic Data

All PK concentrations and parameters will be listed.

Summary tables, arithmetic mean (+ standard deviation [SD]) figures, overlaying individual figures, and individual figures by appropriate treatment and time postdose will be provided for plasma PK concentrations. All figures will be produced on both linear-linear and linear-logarithmic scales, with the exception of figures across all days, which will be produced on the linear-linear scale only. The +SD bars will only be displayed on the linear-linear scale.

Summary tables by appropriate treatment will be provided for all PK parameters, with the exception of diagnostic regression-related PK parameters.

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}. Where subjects tested positive for COVID-19, data will only be listed where available.

If the actual time of sample collection deviates from the allowed PK sample collection window, the plasma concentration will be flagged and excluded from the summary statistics.

Individual concentrations deemed to be anomalous will be flagged in the listings and excluded from the summary statistics.

For plasma concentration data the following rules will apply:

- Values that are BLQ will be set to 0 for the calculation of summary statistics.
- Arithmetic mean or median values that are BLQ will be presented as 0.

For PK parameters the following rule will apply:

Geometric mean and coefficient of variation will not be calculated for t_{last} and t_{max}

8.5.3. Pharmacokinetic Statistical Methodology

No inferential statistical analyses are planned.

8.6. Safety and Tolerability Assessments

8.6.1. Adverse Events

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 24.0 (or higher if a new version is issued during the study; see the DMP for more details).

A treatment-emergent adverse event (TEAE) will be defined as an AE that starts during or after dosing on Day 1, or starts prior to dosing on Day 1 and increases in severity after each dosing.

A treatment related TEAE will be defined as a TEAE with a relationship of possibly related or related to the study treatment, as determined by the investigator.

All AEs will be listed. In addition to the data recorded in the database, the listings will include derived onset time and duration. Onset time will be calculated from the time of dosing for TEAEs only. Where the last associated dose is referring to the last dose received prior to the start of a TEAE.

The frequency of subjects with TEAEs and the number of TEAEs will be summarized for the following categories:

- TEAEs (overall, serious, leading to discontinuation, and leading to death) by treatment
- TEAEs by severity and treatment
- Treatment-related TEAEs (overall, serious, leading to discontinuation, and leading to death) by treatment
- Treatment-related TEAEs by severity and treatment

The frequency of subjects will be summarized separately for TEAEs and treatment-related TEAEs by the following:

- System organ class, preferred term, and treatment
- Preferred term and treatment

For the AE data the following rules will apply:

- For the derivation of treatment-emergent status (applicable to all AEs): The collecting time window for AEs will be from after the time of signing the ICF until end of study (EOS)
- For the derivation of treatment-emergent status (applicable to all AEs): If the start date/time of an AE is incomplete or missing, an AE will be assumed to be a TEAE, unless the incomplete start date/time or the end date/time indicates an AE started

before dosing. For the derivation of treatment-related status (applicable to TEAEs only): If the study treatment relationship for a TEAE is missing, a TEAE will be assumed to be a treatment-related TEAE.

- For the derivation of onset time (applicable to TEAEs only): If the start date/time of a TEAE is missing, onset time will not be calculated. If the start date/time of a TEAE is incomplete, where possible, the minimum possible onset time will be calculated and presented in '≥DD:HH:MM' format (eg, if the date/time of dosing is 01MAY2019/08:00 and recorded start date/time of a TEAE is 03MAY2019, then the minimum possible onset time will be calculated by assuming a TEAE started at the first hour and minute of 03MAY2019 [03MAY2019/00:00], thus will be presented as onset time ≥01:16:00 in the listing). If the start date of a TEAE is the same as the date of dosing but the start time of a TEAE is missing, an onset time will be presented as '≥00:00:01'. Any clock changes will be accounted for in the derivation.

- For the derivation of duration (applicable to all AEs): If the end date/time of an AE is missing, duration will not be calculated. If the start or end date/time of an AE is incomplete, where possible, the maximum possible duration will be calculated and presented in '≤DD:HH:MM' format (eg, if the start of an AE date/time is 01MAY2019/08:00 and its recorded end date/time is 03MAY2019, then the maximum possible duration will be calculated by assuming an AE ended at the last hour and minute of 03MAY2019 [03MAY2019/23:59], thus will be presented as duration ≤02:15:59 in the listing). Any clock changes will be accounted for in the derivation.

- For the calculation of TEAE summary statistics: If the severity of a TEAE is missing, that TEAE will be counted under the 'missing' category.

- For the calculation of TEAE summary statistics: Counting rules for TEAEs: The collecting time window for TEAEs will be from the time of dosing on Day 1 until study completion (Day 1~EOS).

    - For CBP-307 0.05mg dose, only count/collect/record TEAEs that starts or increases in severity during the dosing level of CBP-307 0.05mg (from the time of dosing 0.05mg on Day 1 until the start of dosing 0.1mg on Day 2), irrespective of the TEAE's ending time.

    - For CBP-307 0.1mg dose, only count/collect/record TEAEs that starts or increases in severity during the dosing level of CBP-307 0.1mg (from the time of dosing 0.1mg on Day 2 until the start of dosing 0.2mg on Day 3), irrespective of the TEAE's ending time.

    - For CBP-307 0.2mg dose, only count/collect/record TEAEs that starts or increases in severity during the dosing level of CBP-307 0.2mg (from the time of dosing 0.2mg on Day 3 until the start of dosing 0.5mg on Day 7), irrespective of the TEAE's ending time.

- For CBP-307 0.5mg dose, only count/collect/record TEAEs that starts or increases in severity from the time of dosing 0.5mg on Day 7 until study completion (Day 7~EOS), irrespective of the TEAE's ending time.

- For the calculation of TEAE summary statistics: If a subject experienced multiple TEAEs with the same preferred term for the same treatment, this will be counted as 1 TEAE for that treatment under the maximum severity recorded.

8.6.2. Clinical Laboratory Parameters

All clinical laboratory parameters, their changes from baseline will be listed, as applicable; any value outside the clinical reference range will be flagged. Separate listings will be provided for any parameter for which there is any individual subject value outside the respective clinical reference range.

Summary tables by group treatment sequence and timepoint will be provided for clinical chemistry, hematology parameters, their changes from baseline as applicable.

Where data available, shifts from baseline tables will be provided for clinical chemistry and hematology parameters.

Values recorded as $<x$, $\leq x$, $>x$, or $\geq x$ will be displayed in the listings as recorded. For the derivation of listing flags, all calculations, and presentation in the figures, $<x$ and $\leq x$ values will be set to 0, whereas $>x$ and $\geq x$ values will be set to x.

8.6.3. Vital Signs Parameters

All vital signs parameters, their changes from baseline will be listed, as applicable; any value outside the clinical reference range will be flagged.

Summary tables by group treatment sequence and timepoint will be provided for all vital signs parameters, their changes from baseline, as applicable.

Where data available, shifts from baseline tables will be provided for vital sign parameters.

8.6.4. Safety 12-lead Electrocardiogram Parameters

All 12-lead ECG parameters, their changes from baseline, will be listed; any value outside the clinical reference range will be flagged.

Summary tables by group treatment sequence and timepoint will be provided for all 12-lead ECG parameters, their changes from baseline.

Where data available, shifts from baseline tables will be provided for 12-lead ECG parameters.

An outlier analysis will be performed for QT interval corrected for heart rate using Bazett's formula (QTcB) and QT interval corrected for heart rate using Fridericia's formula (QTcF). The analysis will include all individual original, repeat, and unscheduled postdose values.

The maximum postdose values will be summarized by treatment according to the following categories:

- ≤450 ms
- >450 and ≤480 ms (all instances flagged in the listing)
- >480 and ≤500 ms (all instances flagged in the listing)
- >500 ms (all instances flagged in the listing)

The maximum increases from baseline will be summarized by treatment according to the following categories:

- ≤30 ms
- >30 and ≤60 ms (all instances flagged in the listing)

>60 ms (all instances flagged in the listing)

8.6.5. Cardiac Telemetry Monitoring

All cardiac telemetry data will be listed.

Where data available, shifts from baseline tables will be provided for cardiac telemetry monitoring data.

8.6.6. Other Assessments

Medical history will be listed.

All other safety and tolerability assessments not detailed in the above sections such as physical examination and cardiac telemetry monitoring will be listed only.

8.6.7. Safety and Tolerability Statistical Methodology

No inferential statistical analysis is planned.

9. INTERIM ANALYSES

No interim analysis is planned.

10. SIGNIFICANT CHANGES FROM THE PROTOCOL-SPECIFIED ANALYSES

There were no significant changes from the protocol -specified analyses.

11. REFERENCES

1. ICH. ICH Harmonised Tripartite Guideline: Structure and content of clinical study reports (E3). 30 November 1995.

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3. ICH. ICH Harmonised Tripartite Guideline: Statistical principles for clinical trials (E9). 5 February 1998.

4. 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.

5. Ferber G, M Zhou, B Darpo. Detection of QTc effects in small studies--implications for replacing the thorough QT study. Ann Noninvasive Electrocardiol. 2015;20(4):368-377.

6. Huang DP, Chen J, Dang Q, et al. Assay sensitivity in "Hybrid thorough QT/QTc (TQT)" study. Journal of Biopharmaceutical Statistics. 2019;29(2):378-384.

12. APPENDICES

Appendix 1: Document History

Status and Version	Date of Change	Summary/Reason for Changes
Final Version 1.0	NA	NA; the first version.

NA = not applicable

Statistical Analysis Plan (SAP)/Initiation of Programming Approval Form

Type of Approval (select one) : ☒**SAP** ☐ **Initiation of Programming**

Sponsor Name:	Suzhou Connect Biopharmaceuticals		
Sponsor Protocol/ CIP ID:	CBP-307AU002	**Covance Study ID:**	000000216292
SAP text filename:	CBP-307AU002_SAP_V2_Final_10May2022.pdf	**TFL shells filename:**	CBP-307AU002_TFL_V2_Final_10May2022.pdf
Version:	2.0	**Date:**	10May2022

Covance Approval(s):

Lead Statistician

Approval Signature Print Name Job Title Date	 Wei Xiao Biostatistician I approved this document 16 May 2022 8:19 AM -05:00

Sponsor Approval(s):

By signing below when the statistical analysis plan (SAP) is considered final, the signatories agree to the analyses to be performed for this study and to the format of the associated tables, figures, and listings (TFLs). Once the SAP has been signed, programming of the Analysis Dataset Model (ADaM) datasets and TFLs based on these documents can proceed. Any modifications to the SAP text and TFL shells made after signing may result in a work-scope change.

Approval Signature Print Name Job Title Date	Jessie Chen Clinical Project Manager 16 May 2022 DocuSigned by: Jessie Chen Signer Name: Jessie Chen Signing Reason: I have reviewed this document Signing Time: 2022/5/15 | 7:21:46 PM PDT 2F874039156647FCBB57186A19DC391A

Please scan/email completed form(s) to the Lead Statistician listed below:

Printed Name/Title:	Wei Xiao, Biostatistician
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Cardiac Statistical Analysis Plan

A Phase I, Multicenter, Randomized, Double-blind, Double-dummy, Placebo- and Positive-Controlled Study to Investigate the Effects of CBP-307 on the QTC Interval in Healthy Subjects

Protocol: CBP-307AU002

Prepared For:
Suzhou Connect Biopharmaceuticals, Ltd.

Version: 1.0
Authors: Xingchi Wang, MS, Biostatistician I
Hongqi Xue, PhD, Sr. Principal Biostatistician
Date: 11 March 2022

Revision History

Version	Issue Date	Author(s)	Description
Draft 0.1	14 Dec 2021	Xingchi Wang and Hongqi Xue	Initial version for review.
Draft 0.2	10 January 2022	Hongqi Xue	Revised version per comments.
Draft 0.3	24 January 2022	Hongqi Xue	Updated per protocol.
1.0	1 March 2022	Hongqi Xue	Final version.

Table of Contents

1 Abbreviations

Abbreviation	Term/Description
bpm	Beats per minute
CI	Confidence interval
C-QTc	Concentration-QTc
C_{max}	Maximum plasma concentration
Δ	Change-from-baseline
ΔΔ	Placebo-corrected or 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
RR	RR interval of the ECG
SAP	Statistical analysis plan
SD	Standard deviation
SE	Standard error
T_{max}	Time of peak plasma concentration
TQT	Thorough QT

2 Introduction

This statistical analysis plan (SAP) was developed after review of protocol CBP-307AU002 (Version 5.0 dated 12 January 2022) for the study "A Phase I, Multicenter, Randomized, Double-blind, Double-dummy, Placebo- and Positive-Controlled Study to Investigate the Effects of CBP-307 on the QTC Interval 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 Phase I, randomized, double-blind, double-dummy, placebo-controlled, positive-controlled, multi-site, 3-arm study to investigate the effects of therapeutic and supratherapeutic oral doses of CBP-307 on the QTc interval in healthy male and female subjects.

In this study, approximately 68 healthy subjects (at least 30% for each sex) will be randomized into 2 groups (Group 1 and 2) with 34 subjects in each. Group 2 consists of 2 sub-groups (Group 2A and 2B) and each sub-group will be randomized with 17 subjects. Randomized subjects will receive the assigned study drug as a single dose in the morning under fasting conditions on Days 1, 2, 3 to 6, 7, to 15, and on Day 16 (placebo will be administered to all subjects on Day -1).

The following treatments will be administered:

- Starting doses of CBP-307 for titration (0.05 mg on Day 1 followed by an up-titrated dose of 0.1 mg on Day 2)
- A therapeutic dose of CBP-307 (0.2 mg, Days 3 to 6)
- A supratherapeutic dose of CBP-307 (0.5 mg, Days 7 to 15)
- Placebo (matched to moxifloxacin and CBP-307)
- Moxifloxacin (400 mg)

Moxifloxacin will be used as a positive control to determine the assay sensitivity of this study with an expected peak QT effect (placebo-corrected change-from-baseline QTcF; ΔΔQTcF) of 10 to 15 ms.

Potential subjects will be screened to assess their eligibility to enter the study within 27 days prior to the first dose administration. Subjects will be admitted into the study site on Day -2 (or on Day -4 depending on the clinical site's requirement for Coronavirus Disease 2019 [COVID-19] testing) and will remain at the study site until discharge on Day 19.

4 Cardiodynamic ECG Assessment

4.1 ECG and Pharmacokinetic Sample Collection

Continuous Holter ECG recordings will be performed before dosing for 25 hours on Days -1, 1, 6, 15, and 16 and continue through 12 or 24 hours post-dose. Replicate 12-lead ECGs will be extracted from the continuous recording prior to dosing (-30 minutes) and 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours post-dose on each day. At each time point, up to 10 ECG replicates will be extracted from a 5-minute time window (the last 5 minutes of the 15-minute period when the subject is maintained in a supine, quiet position). The ECG effects of CBP-307 will be tested on data from Day -1 (baseline for both groups), Day 6 (therapeutic concentrations versus placebo), and Day 15 (supratherapeutic concentrations versus placebo). Assay sensitivity with moxifloxacin will be evaluated using ECG data collected on Days -1, 1, 15, and 16.

Pharmacokinetic blood samples will be collected at the following time points: prior to dosing and 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours after dosing on each PK sampling day (Days 1, 6, 15, and 16; sample collection windows are as follows: ±2 minutes for 0.5 hours post-dose, ±5 minutes for 1 to 4 hours post-dose, ±10 minutes for 6 to 8 hours post-dose, and ±30 minutes for 12 to 24 hours post-dose). Additional PK samples will be collected at 72 and 96 hours following dosing on Day 15 (sample collection window: ±30 minutes).

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, NY, 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 (EPQT) (see Appendix A for more details). The ECG database will be locked before any statistical analysis is undertaken.

4.2 Cardiodynamic ECG Objectives

For the purpose of this analysis plan, objectives related to ECG assessment are described.

The primary ECG objective of the study is to evaluate the effect of therapeutic and supratherapeutic CBP-307 plasma concentrations on the heart-rate corrected QT interval (QTc) in healthy subjects.

The secondary ECG objectives of the study are:

- To demonstrate assay sensitivity of the study to detect a small QT effect using moxifloxacin as a positive control.
- To evaluate the effects of therapeutic and supratherapeutic multiple oral doses of CBP-307 on HR, PR and QRS intervals, and T-wave morphology.

4.3 Cardiodynamic ECG Endpoints

The primary ECG endpoint of this study is the change-from-baseline QTcF (ΔQTcF).

The secondary endpoints are:

- Change-from-baseline HR and PR and QRS intervals (ΔHR, ΔPR, and ΔQRS);
- Placebo-corrected change-from-baseline HR, QTcF, PR, and QRS (ΔΔHR, ΔΔQTcF, ΔΔPR, and ΔΔQRS);
- Categorical outliers for QTcF, HR, PR, and QRS;
- Frequency of treatment-emergent changes for 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 subjects enrolled in the study who receive at least 1 dose of study treatment (CBP-307, moxifloxacin, or placebo) 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, visit (or day), 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 QTc), data will be summarized including number of subjects (n), mean, median, standard deviation (SD), 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, standard error (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

Analysis Population	Definition
PK population	All subjects who received at least 1 dose of study treatment (therapeutic and supratherapeutic doses of CBP-307, moxifloxacin) and have evaluable PK data of any of the analytes (CBP-307 and moxifloxacin). A subject will be excluded from the PK summary statistics and statistical analysis if the subject has an adverse event of vomiting that occurs at or before 2 times median time to maximum concentration.
QT/QTc population	All subjects who received at least 1 dose of study treatment (therapeutic and supratherapeutic doses of CBP-307, moxifloxacin, or placebo) with measurements at baseline as well as on-treatment with at least 1 post-dose time point with a valid ΔQTcF value. The QT/QTc set will be used for the by-time point, categorical, and morphological analyses of cardiodynamic ECG parameters.
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 ΔQTcF data from the same time point as well as subjects in the QT/QTc population who received placebo. The PK/QTc Set will be used for the concentration-QTc (C-QTc) analysis and assay sensitivity. PK/QTc Set will be defined for CBP-307 and for 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	Each post-baseline time point that has a nonmissing change-from-baseline observation. Defined for all continuous ECG parameters for subjects in the QT/QTc population.
Categorical analysis set	For continouous ECG parameters, each post-baseline time point that has a nonmissing change-from-baseline observation. For all categorical ECG parameters, each evaluable post-baseline time point that has an evaluable record at baseline, i.e., treatment-emergent changes can be assessed. Defined for all subjects in the QT/QTc population.
Concentration-QTc analysis set	All time points in the by-time point population that have a corresponding planned PK collection at the same time point as well as time points in the by-time point analysis with subjects receiving placebo. 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 population.

5.3 Baseline

Baseline for the assessment of the ECG effect of CBP-307 (CBP-307 in Group 1 versus placebo in Groups 2A and 2B) will be time-matched values on Day -1 (Table 3).

For assay sensitivity in Groups 2A and 2B, the following baselines will be used:

- Group 2A: For moxifloxacin administered on Day 1 (defined as Period 1 Day 1), baseline will be Day 16 (defined as Period 1 Day -1), on which subjects are administered placebo. For placebo-correction in this group, placebo values will be derived from Day 15 (defined as Period 2 Day 1) on which subjects are administeredplacebo, and baseline will be obtained on Day -1 (defined as Period 2 Day -1).
- Group 2B: For moxifloxacin administered on Day 16 (defined as Period 2 Day 1), baseline will be Day 1 (defined as Period 2 Day -1), on which subjects are administered placebo. For the placebo-correction in this group, Day -1 (defined as Period 1 Day 1) values will be used as placebo (no treatment) and baseline will be obtained on Day 15 (defined as Period 1 Day -1).

For T-wave morphology and U-wave presence, baseline includes findings observed in any replicates from all time points that constitute baseline.

Table 3 Treatment administration and baseline definition

Day	Treatment Arm	Control Arm	
	Group 1	Group 2A	Group 2B
-1	CBP-307 placebo Baseline for T1, T2, T3	CBP-307 placebo Baseline for T5 and T6; Baseline for T8 moxifloxacin-placebo on Day 15	CBP-307 placebo Baseline for T4, T5 and T6; T8: moxifloxacin-placebo on Day -1;
1	CBP-307 0.05 mg Moxifloxacin-placebo T1: CBP-307 0.05 mg on Day 1	400 mg Moxifloxacin CBP-placebo T7: moxifloxacin on Day 1	Moxifloxacin-placebo CBP-placebo T4: corresponding placebo on Day 1; Baseline for T7 on Day 16
6	CBP-307 0.2 mg T2: CBP-307 0.2 mg on Day 6	CBP-307-placebo T5: corresponding placebo on Day 6	CBP-307-placebo T5: corresponding placebo on Day 6
15	CBP-307 0.5 mg T3: CBP-307 0.5 mg on Day 15	CBP-307-placebo T6: corresponding placebo on Day 15 T8: moxifloxacin-placebo on Day 15	CBP-307-placebo T6: corresponding placebo on Day 15; Baseline for T8 moxifloxacin-placebo on Day -1
16	CBP-307 placebo Moxifloxacin-placebo (not analyzed)	CBP-307 placebo Moxifloxacin-placebo Baseline for T7 on Day 1	CBP-307 placebo 400 mg Moxifloxacin T7: moxifloxacin on Day 16

Note: To facilitate analysis, the nested crossover design for the moxifloxacin/placebo comparison for assay sensitivity needs to be coded following a 2-period crossover study design

with time-matched baseline on Day -1 in each period. The observations from Groups 2A and 2B will be relabeled (see Table 4). For example, for Group 2A, the moxifloxacin-placebo arm, baseline is Day 16 for the moxifloxacin day (Day 1) and baseline is Day -1 for the placebo day (Day 15). Therefore, Day 1 and Day 15 will be coded as Day 1, and Day -1 and Day 16 will be coded as Day -1.

Table 4 Period definition for Group 2A and 2B

			Treatment Days (per Table 3)			
Sub-group	Treatment	Period	-1	1	15	16
2A	Moxi/Placebo	Period 1 (Moxi)		Day 1		Day -1
		Period 2 (Placebo)	Day -1		Day 1	
2B	Placebo/Moxi	Period 1 (Placebo)	Day 1		Day -1	
		Period 2 (Moxi)		Day -1		Day 1

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]$^{1/3}$.

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

5.5 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 will be calculated for CBP-307 on Day 1, 6, and 15, for moxifloxacin and for placebo for CBP-307, and placebo for moxifloxacin at each post-dose time point. LS mean, SE, and 2-sided 90% CI of ΔΔQTcF will be calculated for each CBP-307 dose group and for moxifloxacin group 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*: Model-predicted mean ΔQTcF in each CBP-307 dose group (or moxifloxacin group) minus model-predicted mean ΔQTcF in the respective 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), decile 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 each CBP-307 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 decile plot(s), as described in Section 8.

6 Analysis

6.1 Concentration-QTc Analysis (Primary Analysis)

The relationship between CBP-307 plasma concentrations and change-from-baseline QTcF (ΔQTcF) will be quantified using a linear mixed-effects modeling approach with ΔQTcF as the dependent variable, time-matched concentrations of CBP-307 as the exploratory variate (0 for placebo), treatment (active=1 or placebo=0), and time (i.e., post-baseline time points on Days 1, 6, and 15: categorical) as fixed effects, and a random intercept and slope for concentrations per subject (Garnett et al[1]). Centered baseline QTcF (i.e., baseline QTcF for individual subject minus the population mean baseline QTcF for all subjects) will be included in this model as an additional covariate. If necessary, a sensitivity analysis will be performed using the model without this term. CBP-307 plasma concentrations below the quantifiable limit at pre-dose 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 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 concentration) and the treatment effect-specific intercept

(defined as the difference between CBP-307 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.

For the assessment of the ECG effect of CBP-307 versus placebo, the time term incorporated into the models (both by-time point analysis and concentration-QTc analysis [or assay sensitivity]) includes the single pre-dose time point and all post-dose time points on Days 1, 6, and 15, and Days 1 and 16 for active versus placebo and moxifloxacin versus placebo, respectively. All times are relative to the time of dosing on that day which is considered the first dose for the assay sensitivity analysis. For the analysis of CBP-307 versus placebo, the first dose of study treatment is on Day 1.

The geometric mean of the individual C_{max} values for subjects in CBP-307 group on each day of Days 1, 6, and 15 will be determined, respectively. The population point estimate and its 2-sided 90% CI for the ΔΔQTcF (i.e., slope estimate × concentration + treatment effect-specific intercept) interval at these geometric mean C_{max} will be obtained. If the upper bound of the 2-sided 90% CI of the predicted ΔΔQTcF effect at the observed geometric mean C_{max} of CBP-307 on Days 6 and 15 is below 10 ms, it will be concluded that CBP-307 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 ΔQTcF values adjusted by population time effect estimated from the model will be used. These individual placebo-adjusted $\Delta QTcF_{i,k}$ ($\Delta\Delta QTcF_{i,k}$) values are derived as the observed individual $\Delta QTcF_{i,k}$ for subject i administered with CBP-307 or placebo at time point k minus the estimated population mean placebo effect at time point k (i.e., time effect). A decile plot of observed drug concentrations and the mean placebo-adjusted ΔQTcF (ΔΔQTcF) and 90% CI at the median concentration within each decile will be given. The regression line presenting the model-predicted ΔΔQTcF (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 6.1.1) and the justification for the choice of the pharmacodynamic model (linear versus nonlinear, Section 6.1.2) as follows.

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 PKPD = PK/QTc population, SUBJID = subject ID, TRT = treatment (1 for CBP-307, and 0 for placebo = 0), TIME = nominal post-baseline time point, CONC= plasma concentration of CBP-307, CBASE = centered baseline QTcF, T1= active dose on Day 1, GeoMeanCmax_1=geometric mean C_{max} for T1, DQTc = ΔQTcF.

Note: ESTIMATE statements will be repeated for active dose groups on Days 6, and 15.

6.1.1 Investigation of Hysteresis

Hysteresis will be assessed based on joint graphical displays of the least squares (LS) mean ΔΔQTcF at each post-baseline time point from the by-time point analysis and the mean concentrations of CBP-307 at the same time points. In addition, hysteresis loops will be given for LS mean ΔΔQTcF from the by-time point analysis and the mean concentrations. Other concentration-QTc models such as a model with an effect compartment may be explored if all of the following 3 conditions are met.

- A QT effect ≥ 10 ms (i.e., LS mean ΔΔQTcF ≥ 10 ms) cannot be excluded in the by-time point analysis in the CBP-307 dose groups on Days 6 and 15
- The mean peak ΔΔQTcF effect is observed at the same post-dose time point in the by-time point analysis in the CBP-307 dose groups on Days 6 and 15
- The difference (delay) between the time to reach the peak QTc effect (ΔΔQTcF) and T_{max} is larger than 1 hour in a consistent way in the CBP-307 dose groups on Days 6 and 15

With the provision stated above, hysteresis will be demonstrated 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.

6.1.2 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 will be produced. Scatter plots of standardized residuals versus concentration, versus model fitted values of ΔQTcF, and versus centered baseline QTcF (if available) will also be produced, as well as box plots of standardized residuals versus nominal time, and versus CBP-307 treatment. Among these plots, the scatter plots of standardized residuals versus concentration, and versus centered baseline QTcF (if available) will include the locally weighted scatter plot smoothing (LOESS, Cleveland[3]) line with an optimal smoothing parameter selected by the Akaike information criterion with a correction (AICC).(Hurvich et al[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.

6.2 Assay Sensitivity

The analysis to show assay sensitivity will be based on the concentration-QTc analysis of the effect on ΔΔQTcF of 400 mg oral moxifloxacin using a similar model as for the primary analysis. That is, the relationship between moxifloxacin plasma concentration and ΔQTcF will be investigated by linear mixed-effects modeling. The model will include ΔQTcF as the dependent variable, moxifloxacin plasma concentration as the explanatory variable (0 for placebo), study treatment (1 for moxifloxacin and 0 for placebo) and time (i.e., post-baseline time point on Day 1

as defined in Table 4 for each period) as fixed effects, and random effects on intercept and slope per subject (Garnett et al[1]). Centered baseline QTcF will be included in this model as an additional covariate. If necessary, a sensitivity analysis will be performed using the model without this term. 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 ΔΔQTcF (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/ΔQTcF 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.

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 T7' TRT 1 CONC &GeoMeanCmax_1 / CL ALPHA=0.1;
RUN;
```

Where PKPD = PK/QTc population, SUBJID = subject number, TRT = treatment (1 for moxifloxacin = 1 and 0 for placebo), TIME = nominal post-baseline time point on Day 1, CONC= plasma concentration of moxifloxacin, CBASE = centered baseline QTcF, T7= moxifloxacin group, GeoMeanCmax_1=geometric mean C_{max} for T7, DQTc = ΔQTcF.

6.3 By-Time Point Analysis

The by-time point analysis for QTcF will be performed for CBP-307 versus placebo. A linear mixed-effects model will be used with ΔQTcF as the dependent variable and time (i.e., post-baseline time point on Days 1, 6, and 15: categorical), treatment (therapeutic dose of CBP-307 on Day 1, therapeutic dose of CBP-307 on Day 6, supratherapeutic dose of CBP-307 on Day 15, and corresponding placebo), and time-by-treatment interaction as fixed effects. Baseline QTcF will be included in this model as an additional covariate. If necessary, a sensitivity analysis will be performed using the model without this term. An unstructured covariance matrix will be specified for the repeated measures at post-baseline time points within subjects. If the model with an unstructured covariance matrix fails to converge, this by-time point analysis will be performed by day separately, and other covariance matrices such as compound symmetry and autoregressive will be considered, in this order, until convergence is achieved. From this analysis, the LS mean, SE, and 2-sided 90% CIswill be calculated for the contrast “CBP-307 versus placebo” at each post-baseline time point on Days 1, 6, and Day 15, respectively.

The by-time point analysis for QTcF will be also performed for moxifloxacin versus placebo at post-baseline time points on Day 1 (as defined in Table 4 for each period). A linear mixed-effects model will be used with ΔQTcF as the dependent variable and time (i.e., post-baseline time points on Day 1: categorical), treatment (moxifloxacin and placebo), period (as defined in Section 5.3 and illustrated in Table 4), sequence (placebo/moxifloxacin or moxifloxacin/placebo), and time-by-treatment interaction as fixed effects. Baseline QTcF will be

included in this model as an additional covariate. If necessary, a sensitivity analysis will be performed using the model without this term. An unstructured covariance matrix will be specified for the repeated measures at post-baseline time points for subject within period. The model will also include a subject-specific random effect. If the model with an unstructured covariance matrix fails to converge, other covariance matrices, such as compound symmetry or autoregressive, will be considered. From this analysis, the LS mean, SE, and 2-sided 90% CIs will be calculated for the contrast "moxifloxacin versus placebo" at each post-baseline time point on Day 1, respectively.

For HR, PR, QRS intervals, the analysis will be based on the change-from-baseline post-dosing values (ΔHR, ΔPR, and ΔQRS). The same (by-time point analysis) model will be used as described for QTcF. 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 QTcF for CBP-307 versus placebo is as follows.

```
PROC MIXED DATA=ECG;
CLASS SUBJID TREAT TIME;
MODEL DQTc=TREAT TIME TREAT*TIME BASE/DDFM=KR;
REPEATED TIME / SUBJECT = SUBJID type = UN;
LSMEANS TREAT*TIME/CL DIFF ALPHA=0.1;
RUN;
```

Where ECG = QT/QTc population, SUBJID = subject number, TREAT = treatment (dose of CBP-307 on Day 1, on Day 6, and on Day 15, and corresponding placebo), TIME = nominal post-baseline time point, BASE = baseline QTcF, and DQTc = ΔQTcF.

The SAS code for the by-time point analysis for QTcF for moxifloxacin versus placebo is as follows.

```
PROC MIXED DATA=ECG;
CLASS SUBJID TREAT TIME PERIOD SEQUENCE;
MODEL DQTc=TREAT TIME TREAT*TIME PERIOD SEQUENCE BASE/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 (moxifloxacin and placebo), TIME = nominal post-baseline time point, PERIOD= period, SEQUENCE = sequence, BASE = baseline QTcF, and DQTc = ΔQTcF.

6.4 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 5).

Table 5 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 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.

6.5 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). 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. For baseline, the category will be deemed as present if observed in any replicates from all time points that constitute baseline.

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 also 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.

6.6 Determination of Sample Size

Approximately 68 healthy subjects (at least 30% for each sex) will be randomized into 2 groups (Group 1 and 2) with 34 subjects in each. Group 2 consists of 2 sub-groups (Group 2A and 2B) and each sub-group will be randomized with 17 subjects.

Determination of sample size for primary analysis

A sample size of 28 evaluable subjects per treatment group will provide more than 94.4% power to exclude that CBP-307 causes more than 10-ms QTc effect at clinically relevant plasma levels, as shown by the upper bound of the 2-sided 90% confidence interval (CI) of the model-predicted QT effect (ΔΔQTcF) at the observed geometric mean C_{max} of CBP-307 in the study. This power is estimated approximately using a 2-sample t-test. The calculation assumes a 1-sided 5% significance level, an underlying effect of CBP-307 of 3 ms and a standard deviation (SD) of the ΔQTcF of 8 ms for both CBP-307 and placebo treatment groups. Note that this calculation is conservative, since it does not take into account any gain in precision due to the use of all data of each subject with the help of a linear mixed-effects model. The concentration-QTc analysis method is supported by Darpo et al[5] and Ferber et al[6], and consistent with the experiences from 25 recent TQT studies.

Determination of sample size for assay sensitivity analysis

To demonstrate assay sensitivity with concentration-QTc analysis, it has to be shown that the ΔΔQTcF of a single dose of 400 mg moxifloxacin exceeds 5 ms (i.e., the lower bound of the 2-sided 90% CI of the predicted QTc effect [ΔΔQTcF] 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 exposure-response analysis was 1.24 ms. The within-subject SD of ΔQTcF in the referred study was 5.4 ms based on the by-time point 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 0.64 required to guarantee a power of at least 95% in a paired t-test situation with a sample size of 28 evaluable subjects. In other words, based on this calculation, a power of at least 95% will be obtained as long as the variability of the ΔQTcF, as measured by its within-subject SD from the by-time point analysis, does not exceed 6.9 ms (ie, 128% [= 0.82/0.64] 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 (Huang et al[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.
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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
14.1.1.1	Baseline values of ECG parameters with descriptive statistics for CBP-307 analysis	n, mean, SD, 90% CI, median, minimum, and maximum from descriptive summary will be given by treatment for each ECG parameter (Section 5.3).
14.1.1.2	Baseline values of ECG parameters with descriptive statistics for moxifloxacin analysis	n, mean, SD, 90% CI, median, minimum, and maximum from descriptive summary will be given by treatment for each ECG parameter (Section 5.3).
14.1.2.1	Observed values of QTcF with descriptive statistics for CBP-307 analysis	n, mean, SD, 90% CI, median, minimum, and maximum from descriptive statistics will be given by treatment and post-baseline time point (Section 6.3).
14.1.2.2	Observed values of QTcF with descriptive statistics for moxifloxacin analysis	n, mean, SD, 90% CI, median, minimum, and maximum from descriptive statistics will be given by treatment and post-baseline time point (Section 6.3).

Number	Title	Comments
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 for CBP-307 analysis	n, LS mean, SE, and 90% CI from the statistical modeling will be given by treatment and time point (Section 6.3).
14.1.4.1-14.1.4.4	Change-from-baseline QTcF, HR, PR, and QRS (ΔQTcF, ΔHR, ΔPR, and ΔQRS) at each time point for moxifloxacin analysis	n, LS mean, SE, and 90% CI from the statistical modeling will be given by treatment and time point (Section 6.33).
14.1.5.1-14.1.5.4	Placebo-corrected change-from-baseline QTcF, HR, PR, and QRS (ΔΔQTcF ΔΔHR, ΔΔPR, and ΔΔQRS) at each time point for CBP-307 analysis	LS mean, SE, and 90% CI from the statistical modeling will be given by treatment and time point (Section 6.33).
14.1.6.1-14.1.6.4	Placebo-corrected change-from-baseline QTcF, HR, PR, and QRS (ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS) at each time point for moxifloxacin analysis	LS mean, SE, and 90% CI from the statistical modeling will be given by treatment and time point (Section 6.33).
14.1.7.1	QTcF outliers per absolute category for CBP-307 analysis	Number (%) of subjects and time points with QTcF > 450 and ≤ 480 ms, > 480 and ≤ 500 ms, or > 500 ms by treatment (Section 6.4).
14.1.7.2	QTcF outliers per absolute category for moxifloxacin analysis	Number (%) of subjects and time points with QTcF > 450 and ≤ 480 ms, > 480 and ≤ 500 ms, or > 500 ms by treatment (Section 6.44).
14.1.8.1	QTcF outliers per change-from-baseline category for CBP-307 analysis	Number (%) of subjects and time points with ΔQTcF > 30 and ≤ 60 ms, or > 60 ms by treatment (Section 6.4).
14.1.8.2	QTcF outliers per change-from-baseline category for moxifloxacin analysis	Number (%) of subjects and time points with ΔQTcF > 30 and ≤ 60 ms, or > 60 ms by treatment (Section 6.44).
14.1.9.1	Categorical analyses for HR, PR, and QRS for CBP-307 analysis	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 6.4).

Number	Title	Comments
14.1.9.2	Categorical analyses for HR, PR, and QRS for moxifloxacin analysis	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 6.4).
14.1.10.1	T-wave morphology and U-wave presence across treatment groups: treatment-emergent changes for CBP-307 analysis	Number (%) of subjects and time points falling into each of the T-wave and U-wave categories will be given by treatment (Section 6.5).
14.1.10.2	T-wave morphology and U-wave presence across treatment groups: treatment-emergent changes for moxifloxacin analysis	Number (%) of subjects and time points falling into each of the T-wave and U-wave categories will be given by treatment (Section 6.55).
14.1.11.1	Concentration-QTc analysis of CBP-307 and associated ΔQTcF prolongation	Fixed-effect estimations and corresponding *P* values will be given (Section 6.1).
14.1.11.2	Assay sensitivity analysis of moxifloxacin and associated ΔQTcF prolongation	Fixed effect estimations and corresponding *P* values will be given (Section 6.2).
14.1.12.1	Predicted ΔΔQTcF interval at geometric mean peak CBP-307 concentration	Section 6.1. Referred to above as a "Concentration-QTc prediction table."
14.1.12.2	Predicted ΔΔQTcF interval at geometric mean peak moxifloxacin concentration	Section 6.2. Referred to above as a "Concentration-QTc prediction table."

8.2 Figures

Number	Title	Comments
14.2.1.1	Observed QTcF across time points for CBP-307 analysis	Mean and 90% CI from descriptive summary will be given by treatment (Section 6.33).
14.2.1.2	Observed QTcF across time points for moxifloxacin analysis	Mean and 90% CI from descriptive summary will be given by treatment (Section 6.33).
14.2.2.1-14.2.2.4	Change-from-baseline QTcF, HR, PR, and QRS (ΔQTcF, ΔHR, ΔPR, and ΔQRS) across time point for CBP-307 analysis	LS mean and 90% CI from the statistical modeling will be shown by treatment (Section 6.3).

Number	Title	Comments
14.2.3.1-14.2.3.4	Change-from-baseline QTcF, HR, PR, and QRS (ΔQTcF, ΔHR, ΔPR, and ΔQRS) across time point for moxifloxacin analysis	LS mean and 90% CI from the statistical modeling will be shown by treatment (Section 6.3).
14.2.4.1-14.2.4.4	Placebo-corrected change-from-baseline QTcF, HR, PR, and QRS (ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS) across time point for CBP-307 analysis	LS mean and 90% CI from the statistical modeling will be shown by treatment (Section 6.33).
14.2.5.1-14.2.5.4	Placebo-corrected change-from-baseline QTcF, HR, PR, and QRS (ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS) across time point for moxifloxacin analysis	LS mean and 90% CI from the statistical modeling will be shown by treatment (Section 6.33).
14.2.6.1	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).
14.2.6.2	QTcF-RR decile plot by treatment	QTcF-RR decile plots with linear mixed-effects line and 90% CI will be given (Section 5.4).
14.2.7.1	Mean CBP-307 plasma concentrations over time	Section 6.1.
14.2.7.2	Mean moxifloxacin plasma concentration over time	Section 6.2.
14.2.8.1-14.2.8.3	CBP-307 plasma concentrations and ΔΔQTcF over time	Section 6.1.1.
14.2.9.1-14.2.9.3	Hysteresis plot of CBP-307 plasma concentration and ΔΔQTcF connected in temporal order	Section 6.1.1.
14.2.10.1	Scatter plot of observed CBP-307 plasma concentrations and ΔQTcF with simple linear regression line and LOESS regression	Scatter plot of observed ΔQTcF versus concentration with LOESS line and 90% CI and simple regression line (Section 6.1.2).
14.2.10.2	Scatter plot of observed moxifloxacin plasma concentrations and ΔQTcF with simple linear regression line and LOESS regression	Scatter plot of ΔQTcF versus concentration with LOESS line and 90% CI and simple regression line (Section 6.2).

Number	Title	Comments
14.2.11.1	Scatter plot of observed CBP-307 plasma concentrations and estimated placebo-adjusted ΔQTcF	Scatter plot of placebo-adjusted ΔQTcF versus concentration with linear mixed-effects regression line and 90% CI of ΔΔQTcF (Section 6.1). Referred to above as "Scatter plot for concentration-QTc model(s)."
14.2.11.2	Scatter plot of observed moxifloxacin plasma concentrations and estimated placebo-adjusted ΔQTcF	Scatter plot of placebo-adjusted ΔQTcF versus concentration with linear mixed-effects regression line and 90% CI of ΔΔQTcF (Section 6.2). Referred to above as "Scatter plot for concentration-QTc model(s)."
14.2.12.1	Model-predicted ΔΔQTcF (mean and 90% CI) and estimated placebo-adjusted ΔQTcF (mean and 90% CI) across deciles of CBP-307 plasma concentrations	Section 6.1. Referred to above as "Decile plots."
14.2.12.2	Model-predicted ΔΔQTcF (mean and 90% CI) and estimated placebo-adjusted ΔQTcF (mean and 90% CI) across deciles of moxifloxacin plasma concentrations	Section 6.2. Referred to above as "Decile plots."
14.2.13.1	Model-predicted ΔΔQTcF interval at geometric mean peak CBP-307 concentrations	Section 6.1. Referred to above as "Prediction plots."
14.2.13.2	Model-predicted ΔΔQTcF interval (mean and 90% CI) at geometric mean peak moxifloxacin concentrations	Section 6.2. Referred to above as "Prediction plots."
14.2.14.1	Scatter plot of standardized residuals versus fitted values for CBP-307	Section 6.1.2.
14.2.14.2	Scatter plot of standardized residuals versus fitted values for moxifloxacin	Section 6.2.
14.2.15.1	Scatter plot of standardized residuals versus concentrations with LOESS for CBP-307	Section 6.1.2.

Number	Title	Comments
14.2.15.2	Scatter plot of standardized residuals versus concentrations with LOESS for moxifloxacin	Section 6.2.
14.2.16.1	Scatter plot of standardized residuals versus centered baseline QTcF with LOESS for CBP-307	Section 6.1.2.
14.2.16.2	Scatter plot of standardized residuals versus centered baseline QTcF with LOESS for moxifloxacin	Section 6.2.
14.2.17.1	Box plot of standardized residuals versus nominal time for CBP-307	Section 6.1.2.
14.2.17.2	Box plot of standardized residuals versus nominal time for moxifloxacin	Section 6.2.
14.2.18.1	Box plot of standardized residuals versus treatment for CBP-307	Section 6.1.2.
14.2.18.2	Box plot of standardized residuals versus treatment for moxifloxacin	Section 6.2.
14.2.19.1	Normal Q-Q plot of standardized residuals for CBP-307	Section 6.1.2.
14.2.19.2	Normal Q-Q plot of standardized residuals for moxifloxacin	Section 6.2.
14.2.20.1	Normal Q-Q plots of the estimated random effects for CBP-307	Section 6.1.2.
14.2.20.2	Normal Q-Q plots of the estimated random effects for moxifloxacin	Section 6.2.

8.3 Listings

Number	Title	Comments
16.2.1.1.1-16.2.1.1.4	QTcF, HR, PR, and QRS intervals - observed and change-from-baseline values for CBP-307 analysis	Section 6.33 for CBP-307 analysis.
16.2.1.2.1-16.2.1.2.4	QTcF, HR, PR, and QRS intervals - observed and change-from-baseline values for moxifloxacin analysis	Section 6.3 for moxifloxacin analysis.
16.2.2.1	T-wave morphology and U-wave presence for CBP-307 analysis	Section 6.55 for CBP-307 analysis.
16.2.2.2	T-wave morphology and U-wave presence for moxifloxacin analysis	Section 6.5 for moxifloxacin analysis.
16.2.3.1	ΔQTcF and time-matched CBP-307 concentrations for each subject	Data for concentration-QTc analysis (Section 6.1).
16.2.3.2	ΔQTcF and time-matched moxifloxacin concentrations for each subject	Data for assay sensitivity (Section 6.2).

9 Approvals

ERT

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13-Mar-2022 | 19:48:41 PDT

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Date

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 cardiac safety specialist. All low confidence beats will be reviewed manually by an ERT cardiac safety specialist 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 cardiac safety specialist 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.

16.1.9.2. Quality Tolerance Limit Definitions

Parameter	Justification for Parameter	Unit Tolerance
Minimum number of evaluable subjects needed.	A shortfall in the overall number of subjects could have had a significant impact on interpretation of the primary endpoint because of limited/insufficient exposure.	N:64