A Randomized, Positive and Placebo-Controlled Study to Evaluate the Effects of HSK3486 Administration on Cardiac Repolarization in Healthy Participants

Statistical Analysis Plan

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[redacted]

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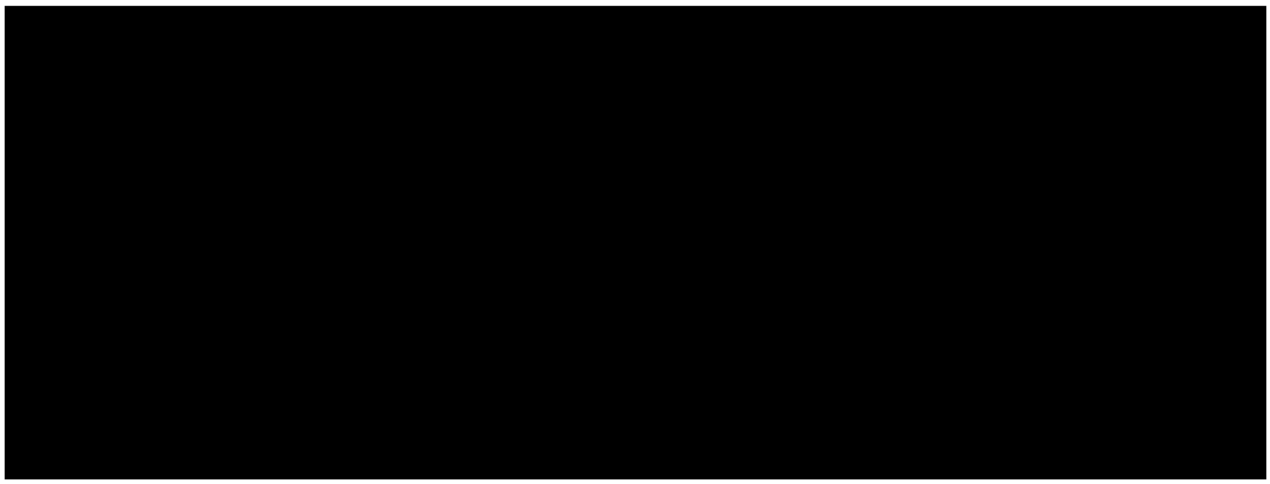

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Approved by (Sponsor)

[redacted]

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Revision History

Version Number	Effective Date	Amendment
1.0	April 30, 2024	First Finalization
1.1	May 31, 2024	6.2.1 Baseline "For 12-lead ECG, baseline value will be the last non-missing value recorded prior to the dosing of each period." revised as "For 12-lead ECG, the last timepoint with non-missing value prior to the dosing of each period will be considered to derive baseline value. The mean of available values at this time point will be used as baseline and the best result of overall ECG interpretation at this time point will be chosen as baseline (Normal > Not Clinically Significant > Clinically Significant)." 6.11.5. Electrocardiogram (12-lead ECG) "The mean values of Heart rate, PR interval, QRS duration, QT interval, QTcF will be calculated and summarized using descriptive statistics by study product at each measurement time point. Descriptive statistics of the change from baseline to each post-baseline time will also be provided. 12-lead ECG baseline value will be the mean value of last timepoint with non-missing recorded prior to the dose of each period." revised as "The mean values of Heart rate, PR interval, QRS duration, QT interval, QTcF will be calculated and summarized using descriptive statistics by study product at each measurement time point. Descriptive statistics of the change from baseline to each post-baseline time will also be provided." 6.11.1 Adverse Events "For all analyses of TEAEs, when calculating the number and incidence of AEs (i.e., on a per subject basis) in a given AE class (MedDRA category), AE is counted only once in that category (SOC or PT)

		at the highest severity." revised as "For all analyses of TEAEs, when calculating the number and incidence of AEs (i.e., on a per subject basis) in a given AE class (MedDRA category), AE is counted only once regardless of the number of events."

List of Abbreviations

Abbreviation	Definition
AE	Adverse Event
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the Curve
BLQ	Below the Quantification Limit
BMI	Body Mass Index
CL	Apparent Total Clearance
C_{max}	Maximum Concentration
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of Variation
ECG	Electrocardiogram
eCRF	Electro-Case Report Form
GCV	Geometric Coefficient of Variation
GM	Geometric Mean
h	Hour
ICH	International Council for Harmonisation of TechnicalRequirements for Pharmaceuticals for Human Use
ITT	Intention-To-Treat
kg	Kilogram
LLOQ	Lower Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
min	Minute
mL	Mini Liter
MRT	Mean Residence Time
NCA	Non-Compartmental Analysis
NMPA	National Medical Products Administration
ONR	Outside Normal Range
PK	Pharmacokinetic

Abbreviation	Definition
PKCS	Pharmacokinetic Concentration Set
PKPS	Pharmacokinetic Parameter Set
PN	Preferred Name
PT	Preferred Term
QRS	The Time of Ventricular Depolarization
QT	The Time from Start of the Q Wave to The End of The T wave
QTc	Corrected QT Interval
QTcF	Corrected QT Interval of the ECG Using Fridericia's Formula
QTcI	Individual-specific QT Correction
SAE	Serious Adverse Events
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operation Procedure
SS	Safety Analysis Set
SUSAR	Suspected Unexpected Serious Adverse Reaction
$t_{1/2z}$	Half Life
TEAE	Treatment-Emergent Adverse Event
T_{max}	Time of Maximum Concentration
V_z	Apparent Volume of Distribution
V_{ss}	Volume of Distribution at Steady-state
λ_z	Terminal Elimination Rate Constant.

1. Introduction

This document presents the Statistical Analysis Plan (SAP) for the protocol "A Randomized, Positive and Placebo-Controlled Study to Evaluate the Effects of HSK3486 Administration on Cardiac Repolarization in Healthy Participants". This SAP summarizes the study design, objectives and provides outcome definitions and statistical methods for the analysis of demographics, pharmacokinetic and safety endpoints for the study. Changes made to the SAP after the SAP is signed but prior to study unblinding will be documented in the clinical study report (CSR).

This SAP is based on the protocol version 3.1, dated April 25, 2024. The table, listing and figure shells are supplied in a separate document.

2. Study Objectives

Primary Study Objective:

To assess the effects of a single IV bolus of HSK3486 single dose on cardiac repolarization (QTc interval of the electrocardiogram) for healthy subjects.

Secondary Study Objective:

To assess the effects of a single IV bolus of HSK3486 on other ECG parameters in healthy subjects (relative to a moxifloxacin hydrochloride tablet positive control and to placebo).

To demonstrate assay sensitivity of the study using oral moxifloxacin hydrochloride tablet 0.4 g single dose as a positive control.

To evaluate the safety and tolerability of a single IV bolus of HSK3486 in healthy subjects.

To evaluate the pharmacokinetic (PK) characteristics of HSK3486 and its metabolites (if applicable) after a single IV bolus of HSK3486 for healthy subjects.

3. STUDY DESIGN

3.1. Overall Design

This is a single-center, randomized, double-blind (except moxifloxacin hydrochloride tablet), placebo and positive controlled study with a 6-sequence, three-period crossover design in healthy subjects. A blind design is used for administration of HSK3486 and placebo, and an open-label design is used for moxifloxacin hydrochloride tablet.

48 subjects who meet all inclusion criteria and none of the exclusion criteria will be randomized in equal proportion to one of 6 dosing sequences (see Table 1 for dosing sequences), and the study procedure consists of 3 periods, with washout period of 5-7 days between periods.

Table 1 Dosing Sequences

Dosing Sequences	Period I	Period II	Period III
1	C	B	A
2	A	B	C
3	B	C	A
4	C	A	B
5	A	C	B
6	B	A	C

- A: Placebo (HSK3486 simulator) (Negative control)
- B: moxifloxacin hydrochloride tablet 0.4g (Positive control)
- C: HSK3486 0.4 mg/kg (Study drug)

3.2. Rationale for The Study

3.2.1. Scientific Basis for Study Design

Based on the ICH E14 Guideline[1], The clinical study design is single-center, randomized, blinded (except moxifloxacin hydrochloride tablet) , placebo and positive-controlled, 6-sequence, 3-period crossover will be conducted.

- Crossover design can eliminate the inter-individual variability of endpoint indicators and effectively control the demand for sample size;
- Randomization in the crossover design eliminates confounding factors such as periodic and sequential effects;
- HSK3486 and placebo will be administered in a blinded design, in order to eliminate confounding factors leading to bias in the dosing process and safety evaluation process;
- A placebo-controlled design will be used to distinguish between observed effects related to HSK3486 or other clinical study conditions;
- Moxifloxacin hydrochloride tablet will be selected as a positive control to effectively verify the sensitivity;
- Healthy subjects will be selected for the study population to eliminate the effect of variables such as concomitant medications and concomitant diseases of the subject on ECG parameters;

- The plasma concentration of HSK3486 was characterized by three-phase elimination, with corresponding half-lives of 0.537 minutes (0.11-1.34 minutes) ($t_{1/2}$, α), 6.26 minutes (3.97-10.2 minutes) ($t_{1/2}$, β), and 105 minutes (38.3-279 minutes ($t_{1/2}$, γ). The half-life of moxifloxacin hydrochloride was 11.5–15.6 h in healthy subjects after a single oral dose. Therefore, the washout period between treatment periods is set as 5-7 days, longer than 7 times the drug elimination half-life;

- Since significant hysteresis was observed in the concentration-QTc model used in the prior pooled analysis, this study will utilize the by-time point analysis as the primary analysis;

- In consideration of the large heart rate effect observed in the previous trials, the individual QT correction method (QTcI) will be used as the primary QT correct method.

3.2.2. Dose Selection Rationale

Based on the recommended maximum clinical dose of 0.4 mg/kg for intravenous bolus injection of HSK 3486 for sedation and anesthesia during non-endotracheal intubation surgery/procedures, induction and maintenance of general anesthesia, and sedation during mechanical ventilation during intensive care, approved by the National Medical Products Administration (NMPA), and the PK profile of HSK3486 drug without significant accumulation, the therapeutic dose of 0.4 mg/kg will be determined and a single dose regimen will be adopted.

A Thorough QT/QTc study, as described in the ICH E14 guidance[1], is generally performed using a supratherapeutic dose of a drug to assess the ECG effects of a supratherapeutic exposure (C_{max}) in order to be able to inform the ECG effects of the drug not only at the mean therapeutic exposure expected in typical participants, but also for subsets of participants who, due to intrinsic or extrinsic factors, might be expected to have an exposure substantially larger than the mean exposure at the maximum clinical dose. However, due to HSK3486's sedative properties, it is not feasible to dose healthy participants with a dose that achieves supratherapeutic exposure, as this would likely produce significant respiratory depression requiring intubation and mechanical ventilation, which would make QT assessments uninterpretable. In summary, no supratherapeutic dose will be designed in the dosing regimen.

3.2.3. Sample Size

Forty eight (48) participants will be enrolled in this study, with the expectation that at least 42 evaluable participants will have data from all treatment periods. Moxifloxacin will be included as a positive control to validate the sensitivity of the assay to detect small increases from baseline QTcF duration[2].The sample size power calculations are detailed in Section 9.1 in the protocol version 3.1.

3.2.4. Randomization and Blinding

The study is randomized and blinded (except moxifloxacin hydrochloride tablet).

Randomization

The study is randomized, and randomization table are generated by statistician using SAS version 9.4 or higher. 48 subjects will be equally randomized to one of 6 dosing sequences (as shown in Table 1) with each sequence consisting with 8 subjects and 3 periods. Subject randomization table will be generated by an independent unblinded statistician.

For subjects who have signed the informed consent form (ICF), the investigator will assign the screening number of subject identification according to the chronological order of signing the ICF. The coding principle of the screening number of subjects in this clinical trial is S plus three-digit Arabic number, and if there is no digits in the hundreds and tens, "0" should be added. For example, the screening number of the 1st screening subject is S001, and the screening number of the 15th screening subject is S015. After successful screening, subjects scheduled to be enrolled will be assigned random number on Day-2 of the trial in descending order according to screening numbers. The randomization number format is three Arabic number, e.g. 001 for the first subject, 012 for the 12th subject, etc.

Once a randomization number is successfully issued, it cannot be revoked, and the subject randomization number remains unchanged throughout the study. The subject replacement in this study is not allowed, i.e. randomized subjects, regardless of whether they use the investigational product or not, cannot be assigned to other subjects for re-use if the study is terminated for any reason.

Blinding

This trial is a blinded trial of HSK3486 and placebo administration, and subjects and investigators are not informed of which investigational product subjects receive. Moxifloxacin hydrochloride tablets are administered using an open design. Considering that the subject will be anesthetized after HSK3486 administration, there are cases where the blind is broken after administration. To maximize blinding, a blinded trail arm will be established. Blinded investigators do not participate in the implementation process of clinical trials and are only responsible for judging the relevance of AEs to the study product in the trial.

An independent unblinded statistician and personnel will generate a list of drug numbers that correspond one-to-one to the subject randomization table and administer the labeling. This statistician and personnel will not be involved in the clinical trial. The whole blinding process needs to be recorded in writing. Blinding personnel should not participate in other work related to

this clinical trial. Blinding of drugs will need to be completed before the drugs are shipped to the investigational site.

Unblinded personnel will be responsible for maintaining the blinding of the trial and confirming the confidentiality and security of the information.

Emergency letters and emergency unblinding: At the same time as the blinding, the blinding personnel should prepare a sealed set of emergency letters containing the study drug allocation information (one for each subject receiving HSK3486 and placebo in each period, 2 in total). The emergency letters should meet the requirements of double-blind trial design and should be transported to the clinical trial site before the first visit of the first subject. In case of the following emergencies: when serious adverse events or subjects need emergency rescue and/or treatment, the investigator can perform emergency unblinding according to relevant SOP and keep relevant records. All emergency envelopes should be recovered after the completion of the test, and the opening status of emergency envelopes should be checked and confirmed at the data blind review meeting.

If a subject experiences a suspected unexpected serious adverse reaction (SUSAR) that requires reporting to a regulatory authority, the unblinding should be performed by an unblinded drug safety officer while the subject remains blinded and does not need to be withdrawn from the study.

Unblinding: After database lock, the unblinding procedure will be completed by the blinding personnel according to relevant SOPs. The results of unblinding will be submitted to statisticians for statistical analysis. After unblinding, the blinding scheme should be provided to the Sponsor for storage.

In case of group AE or discontinuation of the trial for any reason, the blinding can be broken in advance with the mutual approval of the sponsor and investigator.

3.2.5. End of Study Definition

End of study date is defined as the date of the last visit of the last subject in the study.

A subject is considered to have completed the study if he/she has completed all phases specified in the clinical trial protocol, including the last visit.

4. Endpoints

4.1. Primary Endpoint

The primary endpoint of this clinical trial is the change-from-baseline in QTc interval, corrected for HR using the individual QT correction method (QTcI) – ΔQTcI.

4.2. Secondary Endpoints

The secondary endpoints of this clinical trial are:

- Change-from-baseline HR, QTcF, PR, and QRS intervals (ΔHR, ΔQTcF, ΔPR, and ΔQRS), which will be used as the dependent variable for calculation of model-derived ΔΔHR, ΔΔQTcF, ΔΔPR, and ΔΔQRS for the by-time point analysis, respectively;
- Categorical outliers for QTcI, QTcF, HR, PR, and QRS intervals;
- Treatment-emergent changes in ECG morphology.

4.3. Pharmacokinetic endpoints

PK parameters in plasma will be calculated from HSK3486 and its metabolites concentrations versus time data with non-compartment techniques by Phoenix WinNolin 8.3 or higher.

Pharmacokinetics parameters used for PK evaluation in this trial are as follows: AUC_{0-t}、$AUC_{0-\infty}$、T_{max}、C_{max}、V_{ss}、λ_z、$t_{1/2z}$ and CL, etc.

PK Parameter	Definition
C_{max}	Maximum observed concentration, determined directly from the concentration-time data
T_{max}	Time to reach C_{max}, determined directly from the concentration-time data
AUC_{0-t}	Area under the curve from the time of dosing to the last measurable (positive) concentration. The calculation depends on the Integral method setting. 'Linear Up Log Down' is chosen for integral method: $AUC_{0-t}= AUC_{(0-t)\ up}+ AUC_{(0-t)\ down}$ $AUC_{(i,\ i+1)}=(t_{i+1}-t_i)(C_i+C_{i+1})/2$, $AUC_{(0-t)\ up}$ is sum of $AUC_{(i,\ i+1)}$, $AUC_{(j,\ j+1)}= (t_{j+1}-t_j)(C_j-C_{j+1})/\ln((C_j/C_{j+1})$, $AUC_{(0-t)\ down}$ is sum of $AUC_{(j,\ j+1)}$.

PK Parameter	Definition
$AUC_{0-\infty}$	AUC from time of dosing extrapolated to infinity, based on the last observed concentration $AUC_{0-\infty}=AUC_{0-t}+C_{last}/\lambda_z$
$AUC_{\%Extrap}$	Percentage of $AUC_{0-\infty}$ due to extrapolation from T_{last} to infinity will be calculated as: $AUC_{\%Extrap}=100\times(AUC_{0-\infty}-AUC_{0-last})\ /\ AUC_{0-\infty}$
λ_z	Terminal elimination rate constant
R_{sq_a}	Goodness of fit statistic for the terminal elimination phase, adjusted for the number of points used in the estimation of λ_z
$t_{1/2z}$	Terminal elimination half-life will be calculated as: $t_{1/2z}=\ln 2\ /\ \lambda_z$
$MRT_{0-\infty}$	Mean residence time extrapolated to infinity based on $AUC_{0-\infty}$ $MRT_{0-\infty}=AUMC_{0-\infty}/AUC_{0-\infty}$, $AUMC_{0-\infty}$ is the area under the first moment curve from time 0 to infinity using observed C_{last}.
V_{ss}	Estimate of the volume of distribution at steady-state. Only parent drug. $V_{ss}=MRT_{0-\infty}\times CL$
V_z	Volume of distribution. Only parent drug. $V_z=CL/\lambda_z$
CL	Total body clearance. Only parent drug. $CL=Dose\ /\ AUC_{0-\infty}$

4.4. Safety endpoints

- Adverse Events (AE), Serious Adverse Events (SAE)
- Vital Signs and Physical Examinations
- Clinical Laboratory Evaluations

- Electrocardiogram (12-lead ECG)
- ECG Monitoring
- Observation of Subject's Consciousness
- Pregnancy Test

5. Analysis Population

Analysis Set	Definition
ITT Analysis Set (ITT)	All randomized subjects. The ITT will be used for analyses of demographic and baseline characteristics.
ECG Analysis Set (ECGS)	All randomized subjects who have received at least 1 investigational product (HSK3486, moxifloxacin hydrochloride tablet, or placebo) and had centrally reviewed cardiodynamic ECG measurements at baseline as well as on-treatment with at least 1 post-dose time point with a valid change-from-baseline value for at least 1 ECG parameter. The ECGS will be used for the by-time point, categorical, and morphological analyses of ECG parameters.
Safey Analysis Set (SS)	All randomized subjects who received at least one investigational product (HSK3486, moxifloxacin hydrochloride tablet, or placebo) and have post-dose safety evaluations.
Pharmacokinetics Concentration Analysis Set (PKCS)	All randomized subjects who have received HSK3486 and have at least one valid plasma concentration data after administration during the trial.
Pharmacokinetic Parameter Analysis Set (PKPS)	All randomized subjects who received HSK3486 and have at least one evaluable PK parameter during the trial. Those excluded from PKPS include: a) those who affect PK parameter results due to serious protocol violations or whose parameters cannot be estimated; b) those whose pre-dose concentration is> 5% of C_{max}; c) those who have concomitant medications during the trial and have an impact on PK parameters.

6. Statistical Methodology

6.1. Data Management

Data management procedures, including database design, coding of medical history, adverse events and medications, will be performed by eStart Medical Technology Co., Ltd. Data will be entered into electronic case report form (eCRF) at the study sites. A series of logic and consistency checks will be conducted to ensure accuracy and completeness of the clinical database. Pharmacokinetic data will be electronically transmitted from external vendors. After

database lock, the unblinding procedure will be completed by the independent unblinded biostatistician from eStart Medical Technology Co., Ltd. Please refer to the Data Management Plan for further data management details.

6.2. General Considerations

6.2.1. Baseline

Unless otherwise specified, a baseline value is the last non-missing value recorded prior to the first dose of a study product. For vital signs, baseline value will be the last non-missing value recorded prior to the dosing of each period. If the date and time of the assessment exactly matches the date and time of the study product dosing, the assessment will be considered as the baseline.

For 12-lead ECG, the last time point with non-missing value prior to the dosing of each period will be considered to derive baseline value. The mean of available values at this time point will be used as baseline and the best result of overall ECG interpretation at this time point will be chosen as baseline (Normal> Not Clinically Significant> Clinically Significant).

Each subject's change from baseline will be calculated as the value at the specified time point minus the baseline value for that subject.

6.2.2. Study Day and Day in Period

For a given date (date), the study day is calculated as days since the date of first dose of a study product (firstdose).

Study day=date-firstdose+1, where date ≥ firstdose

Study day=date-firstdose, where date < firstdose

And the day in period is calculated as days since the dosing date of a specific period.

Day in period=date-dosing date of a specific period+1, where date ≥ dosing date of a specific period

Day in period=date- dosing date of a specific period, where date <dosing date of a specific period.

6.2.3. Body Mass Index (BMI)

BMI (kg/m^2) = weight (kg) / [height $(m)^2$].

6.2.4. Missing Data

Unless otherwise specified, missing data other than start date of adverse events (AE), start/end date of concomitant medications (CM) and Concomitant Procedures will not be imputed.

Missing date on Adverse Events

- **Start date**

  - If the start date is completely missing, the start date is set to the date of first dose.
  - If the year is present and the month and day are missing or the year and day are present and the month is missing:

    If year = year of first dose, then set month and day to month and day of first dose.

    If year < year of first dose, then set month and day to December 31.

    If year > year of first dose, then set month and day to January 1st.
  - If the month and year are present and the day is missing:

    If year = year of first dose and

    If month = month of first dose then set day to day of first dose date

    If month < month of first dose then set day to last day of month

    If month > month of first dose then set day to 1st day of month

    If year < year of first dose then set day to last day of month

    If year > year of first dose then set day to 1st day of month
  - For all other cases, set the start date to the date of first dose

Missing date on Concomitant Medications and Concomitant Procedures

- **Start date**

  - If the start date is completely missing, then the start date will not be imputed.
  - If the year is present and the month and day are missing or the year and day are present and the month is missing:

    Set month and day to January 1st.
  - If the year and month are present and the day is missing:

    Set day to 1st day of month.

- **End date**

  - If the end date is completely missing, then the end date will not be imputed.
  - If the year is present and the month and day are missing or the year and day are present and the month is missing:

    Set month and day to December 31.
  - If the year and month are present and the day is missing:

    Set day to last day of the month.

6.2.5. Medical coding

The versions of MedDRA and WHODrug dictionaries used in this clinical trial are listed below.

Term	Dictionary version
Medical history	MedDRA V27.0 or higher
Adverse events	MedDRA V27.0 or higher
Prior and concomitant medications	WHODrug, version March 2024 or higher
Prior and concomitant procedures	MedDRA V27.0 or higher

6.2.6. General Statistical Analysis

Continuous variable will be summarized using number (N), arithmetic mean, standard deviation (SD), median, minimum, and maximum. Summaries of plasma concentration and PK parameters will be introduced in Pharmacokinetics section.

For categorical variables, frequency and percentages of total for each category will be presented; missing values will be treated as a separate category, where appropriate.

If a reported data of a clinical laboratory parameter cannot be used directly for a statistical summary table (e.g., a character string is linked to a numerical value), the data will be converted to a numerical value by programming codes. For example, a data showed as '<10' or '≤ 5' will be converted to '10' or '5' respectively, and a data as '>100' will be converted to '100'. For the list, however, the original data will be retained.

Sort order of listings will be by sequence, subject, parameter, date (or day) and time. If a listing will not follow this sort order, the sort order to be used can be found in the mockup tables or in listing.

SAS® statistical software package, version 9.4, will be used to provide all summary tables, listings, graphs, and statistical analyses.

Decimal places rules are as flows.

Reporting precision		
Descriptive Summaries	Individual Subject Data	Recorded decimal places

	N	0 decimal places
	Mean, Median	Max recorded decimal places+1，no more than 3 decimal places
	Standard Deviation (SD)	Max recorded decimal places +2，no more than 4 decimal places
	Min, Max	Recorded decimal places
	Percentage（%）	1 decimal place
Plasma Concentration and PK Parameters	Individual Subject Data	Recorded decimal places for plasma concentration and 3 significant digits for PK parameters
	N	0 decimal places
	Min, Median, Max	Recorded decimal places for plasma concentration and 3 significant digits for PK parameters
	CV%, GCV%	1 decimal place
	Mean, SD, Geomean, GSD, 25th and 75th percentile	3 significant digits

6.3. Study Subjects

6.3.1. Subject Disposition

Subject disposition will be summarized by sequence and total column, showing the number and percentage of subjects who were screened, failed, enrolled, randomized, discontinued and completed, as well as by subjects in ITT set, in ECG set, in safety set, in PKCS and in PKPS.

Reasons for discontinuation from the study will also be summarized by sequence and total column.

A flow diagram will summarize screening, failure, enrollment, and disposition of subjects by sequence and period, including number of subjects dosed, completed the study and reasons for discontinuation from the study.

Eligibility for each analysis set along with the reasons for exclusion will be listed. Subjects who failed the screening, discontinued from the study will be listed separately.

6.3.2. Protocol Deviations

Major protocol deviations will be summarized by sequence and total column in the ITT population. This table will provide the number and percentage of subjects for each category. All protocol deviations will be presented by a subject-level listing.

6.4. Demographics and Baseline Characteristic

Demographics data (e.g. age, gender, and Ethnicity) and baseline characteristics (height, weight, and BMI, work status, smoking history, drinking history, allergies history) will be summarized by sequence and total column in the ITT population. Other baseline characteristics including cardiac ultrasound, airway assessment, oxygen saturation (pulse), alcohol breath test, infectious disease screening, infectious disease screening, and Urine drug abuse screening (morphine, methamphetamine, ketamine, methylenedioxyamphetamine, tetrahydrocannabinol) will only be listed by subject and sequence.

Individual subjects listing will be presented for all demographic and baseline characteristics.

6.5. Medical History

Medical history will be coded using the Medical Dictionary of Regulatory Activities (MedDRA) classification, version 27.0 or higher. Medical history will be summarized for the ITT population by system organ class (SOC), preferred term (PT), and sequence, total column.

Subjects reporting the same SOC or PT more than once will be counted only once for that SOC or PT.

A listing of medical history will be provided by sequence and subject.

6.6. Prior and Concomitant Medications

Prior medications are those medications taken before the first dose of study product. Concomitant medications are those medications started on or prior to the first administration of study product and ongoing, or those medications started after the first administration of study product .

Prior and concomitant medications will be coded using the World Health Organization (WHODrug, March 2024 or higher). Medications will be summarized by ATC level 2 category, preferred name (PN), and sequence, total column. ATC level 2 category and PN will be presented in decreasing frequency of the total numbers of subjects with medications. Subjects will be counted only once for an ATC level 2 class and PN. For prior medications, the ITT set will be used, while the Safety

set will be used for concomitant medications.

All prior and concomitant medications will be listed.

6.7. Prior and Concomitant Medical Procedures

Prior medical procedures are those conducted before the first dose of study product. Concomitant medical procedures are those procedures started on or prior to the first administration of study product and ongoing, or those procedures started after the first administration of study product.

Prior and concomitant medical procedures will be coded using the MedDRA classification, version 27.0 or higher. Prior and concomitant medical procedures will be summarized by SOC, PT, and sequence, total column.

SOC and PT will be presented in decreasing frequency of the total numbers of subjects with medical procedure. Subjects reporting the same SOC or PT more than once will be counted only once for that SOC and PT.

A listing of prior and concomitant medical procedures will be provided.

6.8. Study product Exposure

Study product exposure data will be presented by sequence and total column in the safety population. A table will present showing the number and percentage of subjects who has administrated study product for each period.

Study product exposure data will be listed by sequence, subject and period.

6.9. Cardiac ECG Analysis

Cardiac ECG analysis will be specified in a standalone analysis plan and attached to this SAP.

6.10. Pharmacokinetics

6.10.1. Data Handling

（1）Sampling time deviation

Blood sample time handling rules are as flows:

- Unless otherwise noted, any PK sample collection earlier or later than scheduled will be considered a time deviation and recorded, even if the deviation is not PK relevant.
- Protocol deviations are considered only if they exceed the allowable maximum time deviation range, which is defined in the table below.

Table 2 Maximum Allowable Time Deviation for PK Blood Sample Collection

Scheduled Sampling Time	Acceptable Deviation Range
0 h（pre-dose）	Within 60min pre-dose
Immediately after the end of dosing	+6 s
1 min	±6 s
2 min≤t≤5 min	±10 s
10 min≤t≤1 h	±1 min
2 h≤t≤6 h	±10 min

Notes: "t" is scheduled sampling time point

The sample concentrations that the actual sampling time exceeds acceptable deviation range of the nominal time will be excluded from the descriptive statistics.

（2）PK Concentration

For descriptive statistics on concentration data, the following rules will be used:

- In descriptive statistics, concentration data below the lower limit of quantitation (BLQ) will be set to zero.
- Descriptive statistics of Mean, SD and CV% will be calculated if at least 1/2 of the post-dosing individual data points are quantifiable (≥LLOQ).
- For BLQ concentrations before dose administration:
  If more than 1/2 of values are BLQ, arithmetic mean (Mean) will not be calculated and considered as zero. Standard Deviation (SD) and Coefficient of Variation (CV%) will be denoted as 'not calculated' (NC). Minimum and maximum value will be reported exactly as the raw data are reported. Median will be reported based on statistical values.
- For BLQ concentrations after dose administration:
  If more than 1/2 of values are BLQ， arithmetic mean (Mean), standard deviation (SD) and coefficient of variation (CV%) will be denoted as 'not calculated' (NC) Minimum and maximum value will be reported exactly as the raw data are reported. Median will be reported based on statistical values.
- Nominal sample times will be used in the categorization of descriptive statistics. If actual sampling time exceed acceptable deviation range of the nominal time, the concentration value at specific sampling time will be excluded from descriptive statistics but only be listed in the listing.

- Nominal times are to be used to display the mean concentration plots, and concentrations will be graphically represented with an arithmetic mean (+SD).
- Actual blood sampling times are to be used to display the individual concentration-time profile, BLQ will be set to missing
- Values below the LLOQ will be reported as BLQ in the listings.
- Concentration value may also be excluded if the concentration is anomalous for any other reason at the discretion of the pharmacokineticist.

（3）NCA Parameters

Plasma PK parameters will be estimated using non-compartmental methods with WinNonlin®, and AUCs will be calculated using linear up / log down method.

The following rules will be used when conducting NCA:

- Actual sampling times will be used for deriving PK parameters.
- For PK parameters calculation: all BLQ values occurring before T_{max} will be replaced by 0, the BLQ concentration after T_{max} will be treated as missing. A non-BLQ data after 2 BLQ data will be treated as BLQ if without sampling analysis issues.
- All estimations of λ_z will be determined using at least three (not including the concentration at T_{max}) last measurable concentration data. Otherwise, the PK Parameters λ_z, R_{sq_a}, $AUC_{0-\infty}$, $AUC_{\%Extrap}$, $t_{1/2z}$, $MRT_{0-\infty}$, CL, V_z and V_{ss} will not be calculated and be flagged "NC".
- The value of λ_z, R_{sq_a}, $AUC_{0-\infty}$, $AUC_{\%Extrap}$, $t_{1/2z}$, MRT, CL, V_z and V_{ss} will only be listed but not be included in the descriptive statistics if $AUC_{\%Extrap}$ exceeds 20% or the adjusted R^2 is ≥0.80.

6.10.2. Descriptive Statistics and Plots of PK Concentration

Based on PKCS, descriptive statistics will be performed for plasma concentrations, which will be summarized at each time point according to scheduled blood sampling time points, including number of observed cases (N), arithmetic mean (Mean), standard deviation (SD), coefficient of variation (CV%), minimum value (Min), maximum value (Max), median value (Median).

Mean and individual concentration-time profiles of HSK3486 will be presented both on linear and semi-log scales. All plasma concentration data will be listed including actual sampling time.

6.10.3. Descriptive Statistics of PK parameters

Based on PKPS, PK parameters of HSK3486 and its metabolites in humans will be summarized, including number of observations (N), arithmetic mean (Mean), standard deviation (SD), coefficient of variation (CV%), minimum (Min), maximum (Max), median (Median), 25th quartile (Q1), 75th quartile (Q3), geometric mean (GM), geometric standard deviation (GSD) and geometric coefficient of variation (GCV%).

Notes:

Particular subjects with the pre-dose value greater than 5% of C_{max} may be excluded from the descriptive statistics of PK parameters.

All events that may affect pharmacokinetic evaluation, such as adverse events or protocol deviations should be evaluated and confirmed before the database lock.

6.11. Safety Analyses

All safety analyses will be conducted in the Safety population.

6.11.1. Adverse Events

Adverse events will be coded using the MedDRA version 27.0 or higher. The severity of adverse events is assessed according to CTCAE 5.0 criteria. TEAEs are defined as AEs that started after first dosing (inclusive) or worsened in severity after dosing.

Adverse events will be accountable to period 1 treatment if they start on or after the start of the administration study treatment in period 1 until the first administration of study treatment in period 2. However, for period 2, adverse events are accountable to period 2 treatment if they start on or after the start of the administration study treatment period 2 until the first administration of study treatment in period 3. TEAEs in period 3 will be counted in the same way. If the start date and time, and other data mean that the event may have occurred in more than one period, then the event will be recorded under more than one period.

For all analyses of TEAEs, when calculating the number and incidence of AEs (i.e., on a per subject basis) in a given AE class (MedDRA category), AE is counted only once regardless of the number of events. Drug related TEAE is defined as possibly, probably or definitely related to study product.

An overall summary table of AEs will include number of events, number and percentage of subjects in each study product who experienced at least one AE of the following categories: any AE, any TEAE, drug related TEAEs, any SAE, any drug related SAE, TEAEs with grade>=3, drug

related TEAEs with grade>=3, TEAEs leading to dose reduced or interrupted, drug related TEAEs leading to dose reduced or interrupted, TEAEs leading to drug permanent withdrawn, drug related TEAEs leading to drug permanent withdrawn, TEAEs leading to study discontinuation, drug related TEAEs leading to study discontinuation, TEAEs leading to death, drug related TEAEs leading to death.

Moreover, all AE categories above (except for any AE) will be tabulated by SOC and PT; Any TEAE, any drug related TEAE will also be tabulated by SOC, PT, and maximum severity (CTCAE 5.0 criteria), respectively.

Individual data listings will be provided for the above by sequence, period (study product), subject.

6.11.2. Clinical Laboratory Evaluations

Summaries of laboratory data will include hematology, urinalysis, chemistry, serum electrolytes, coagulation, thyroid function laboratory parameters. Pregnancy test data will only be listed. Unplanned clinical laboratory evaluations will be listed as well, except when calculating the worst post-baseline value. Only scheduled post-baseline safety measurements will be summarized. Measurements outside of the normal range will be flagged as 'ONR' (outside normal range) in the listings.

Protocol-specified laboratory tests:

laboratory tests	parameters
Hematology	White blood cell count (WBC), neutrophil count (NEU#), lymphocyte count (LYM#), neutrophil percentage (NEU%), lymphocyte percentage (LYM%), monocyte count (MON#), eosinophil count (EOS#), basophil count (BAS#), red blood cell count (RBC), hemoglobin (HGB), platelet count (PLT)
Blood biochemistry (excluding serum electrolytes)	Total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea (UREA), triglycerides (TG), creatinine (enzymatic)(CR), glucose (GLU), creatine kinase (CK)
Urine routine	Urobilinogen (UBG), urinary bilirubin (BIL), ketone body (KET), occult blood (BLD), urinary protein (PRO), nitrite (NIT), urinary glucose (GLU), urinary specific gravity (SG), pH (PH), white blood cells (high power field), red blood cells (high power field)
Coagulation function	Prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FBG), international normalized ratio (INR)
Thyroid function	Thyroid stimulating hormone (TSH), total thyroid hormone (TT4), free thyroxine (FT4), total triiodothyronine (TT3), free triiodothyronine (FT3)
Serum electrolytes	Potassium (K), magnesium (Mg), total calcium (Ca), sodium (Na), chlorine (Cl)

Infectious disease screening [a]	Hepatitis B surface antigen (HBsAg), hepatitis C antibody (anti-HCV), Treponema pallidum antibody (anti-TP), HIV antigen/antibody combination test (HIV combin)
Blood pregnancy test [b]	β-Human Chorionic Gonadotropin (β-HCG)

a. Only during screening.
b. Pregnancy test for female subjects. β-human chorionic gonadotropin higher than 5 mIU/mL is defined as positive blood pregnancy.

Several analyses of the laboratory data will be presented. Descriptive statistics for observed values and values changed from baseline will be summarized by parameter, timepoint and sequence. Meanwhile, serum electrolytes parameters and change from baseline will be summarized by parameter, timepoint and study product. Change from baseline will be calculated for each subject at the specified timepoint as the value at the specific timepoint minus the baseline value. For serum electrolytes, baseline value will be the last non-missing value recorded prior to the dosing of each period.

Only laboratory parameter reported as continuous values will be included in tables of summary statistics.

The number of subjects with clinical laboratory values categorized as normal, abnormal not clinically significant, and abnormal clinically significant, will be tabulated showing worst change from baseline to follow-up (shift tables) for each clinical laboratory analyte by sequence. Similarly, shift tables will be presented for serum electrolytes by study product.

A general detailed subject list of all abnormal laboratory data collected during the study will be provided by sequence, subject, and laboratory parameter.

6.11.3. Vital Signs

Blood pressure (systolic and diastolic), respiration rate, heart rate, and temperature will be summarized using descriptive statistics by study product at each measurement timepoint. Descriptive statistics on change from baseline to each post-baseline time will also be provided. Vital signs baseline value will be the last non-missing value recorded prior to the dose of each period.

Shift tables comparing worst post-baseline clinical significance with baseline clinical significance values (normal, abnormal not clinically significant, and abnormal clinically significant) will be created by study product for vital signs of blood pressure (systolic and diastolic), respiration rate, heart rate, and temperature.

All abnormal vital signs will be listed for all subjects.

6.11.4. Physical Examinations

Shift tables comparing worst post-baseline clinical significance with baseline clinical significance values (normal, abnormal not clinically significant, and abnormal clinically significant) will be created by sequence for each body system.

Abnormal physical examination findings will be presented in by-subject listings.

6.11.5. Electrocardiogram (12-lead ECG)

The mean values of Heart rate, PR interval, QRS duration, QT interval, QTcF will be calculated and summarized using descriptive statistics by study product at each measurement time point. Descriptive statistics of the change from baseline to each post-baseline will also be provided.

Shift tables will be presented to show the number of subjects for the overall ECG interpretation by study product. ECG interpretation categories include: normal, abnormal not clinically significant, and abnormal clinically significant.

A listing of abnormal electrocardiogram data will also be provided.

6.11.6. ECG Monitoring

ECG monitoring including blood pressure, oxygen saturation, heart rate, respiration.

A listing of abnormal ECG monitoring data will also be provided.

6.11.7. Observation of Subject's Consciousness

Consciousness state findings will be listed by subject, sequence and period.

7. Changes to the statistical analyses planned in the protocol

N/A

8. REFERENCES

1. Guidance for Industry. E14: Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs. FDA, October 2005.
2. Florian JA, Tornøe CW, Brundage R, Parekh A, Garnett CE. Population pharmacokinetic and concentration--QTc models for moxifloxacin: pooled analysis of 20 thorough QT studies. J Clin Pharmacol. 2011;51(8):1152-1162.

CLARIO.

CARDIAC STATISTICAL ANALYSIS PLAN

A Randomized, Positive and Placebo-Controlled Study to Evaluate the Effects of HSK3486 Administration on Cardiac Repolarization in Healthy Participants

Protocol: HSK3486-112

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Revision History

Version	Issue Date	Author(s)	Description
Draft 0.1	29 September 2023	[redacted]	Initial version for review
Draft 0.2	29 April 2024	[redacted]	Updated version for review: adding hysteresis adjustment, deleting descriptive analysis for ΔΔ of ECG parameters, and changing sequence numbers of TFLs.
1.0	11 June 2024	[redacted]	Final version.

Table of Contents

1 Abbreviations

Abbreviation	Term/Description
bpm	Beats per minute
CI	Confidence interval
C_{max}	Maximum plasma concentration
Δ	Change-from-baseline
ΔΔ	Placebo-corrected change-from-baseline
ECG	Electrocardiogram
EPQT	Early Precision QT analysis technique
HR	Heart rate
LVEF	Left ventricular ejection fraction
LS	Least squares
ms	Millisecond
oHCM	Obstructive hypertrophic cardiomyopathy
PK	Pharmacokinetic(s)
PR	PR interval of the ECG
QD	Once daily
QRS	QRS interval of the ECG
QT	QT interval of the ECG
QTc	Corrected QT interval
QTcF	Corrected QT interval using Fridericia's formula
QTcI	Optimized HR-corrected QT interval
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

2.1 Background

HSK3486 injectable emulsion is under development by Haisco Pharmaceutical Group Co., Ltd. for the induction and maintenance of sedation/general anesthesia. The primary mechanism of action for HSK3486 is enabling chloride ion influx by enhancing gamma-aminobutyric acid receptor subtype A-mediated ion channels, thereby inhibiting central nervous system activity. HSK3486, the active moiety, possesses a structure similar to propofol. These channels are also major targets of propofol action.

HSK3486 is a single-configuration chiral compound with one R-designated chiral center. HSK3486 has been engineered such that the structural design aims to enhance the pharmacological and physicochemical properties of drug-receptor binding in a systematic manner. HSK3486 induces general anesthesia rapidly and, furthermore, these structural improvements result in increased potency, smaller amount of drug required, less injection site pain, and may reduce the occurrence of side effects caused by propofol.

The following summary of nonclinical and clinical data is based on the Investigator's Brochure (IB) Version 9.0, dated 10 October 2023.

2.2 Nonclinical Pharmacokinetics

Pharmacokinetic (PK) results showed that HSK3486 exposure (area under the concentration time curve [AUC]) in rats and Beagle dogs was linear. No significant gender differences were observed. Clearance rate was found to be much higher than hepatic blood flow, indicating that HSK3486 is a high-clearance drug. No drug accumulation was observed after 7 consecutive administrations of HSK3486 once every other day. In most tissues, HSK3486 was eliminated quickly and drug concentrations in tissues were lower than 10% of corresponding peak concentrations 240 minutes post-dose. Following intravenous (IV) injection of [^{14}C]HSK3486 in Long-Evans rats, total radioactivity in tissues exhibited a two-phase elimination, i.e., rapid elimination within 4 hours post-dose, then a slower elimination phase. At concentrations of 80-1200 ng/mL, binding rates of HSK3486 to human, dog, and rat plasma proteins were 94.9%, 90.8%, and 91.5%, respectively, indicating no evident differences across species. HSK3486 was rapidly metabolized in liver microparticles of humans, monkeys, dogs, rats, and mice.

An *in vitro* metabolism study showed that CYP2B6 was the most important cytochrome P450 (CYP) enzyme subtype involved in HSK3486 metabolism. Although CYP1A2 and CYP2C19 were also involved in the metabolic elimination of HSK3486, they had a lower contribution rate compared with CYP2B6. HSK3486 exhibited moderate inhibition of CYP2B6 and CYP2C19, and did not induce CYP1A2 and CYP2B6. In addition, HSK3486 could induce CYP3A4 at high

concentrations. Hence, it is necessary to consider clinical plasma exposure levels to arrive at further conclusions concerning potential drug-drug interactions.

The PK characteristics of HSK3486; i.e., fast distribution and clearance, are consistent with its PD; i.e., quick onset and fast recovery.

The results of transporter studies showed that HSK3486 was not a substrate of, nor an inhibitor of, transporters P-glycoprotein and breast cancer resistant protein, or solute carrier (SLC) transporters organic anion transporter (OAT)1, OAT3, OCT2, OATP1B1, OATP1B3, MATE1, and MATE2K, suggesting that the potential of drug-drug interactions of HSK3486 in clinical practice is low.

2.3 Nonclinical Cardiac Safety

2.3.1 hERG analysis by Manual Patch-Clamp Electrophysiology

The potential effect of HSK3486 on human ether-à-go-go-related gene (hERG) current amplitude using a CHO cell line transfected with hERG complementary deoxyribonucleic acid (cDNA) and stably expressing hERG channels at 0 (0.3% dimethyl sulfoxide [DMSO]), 0.6, 2, 6 and 20 μM was examined using a manual patch-clamp technique. Results showed that hERG potassium channel current decreased by 1.9%, 5.63%, 7.32%, and 31.63%, respectively. The positive control Cisapride inhibited hERG potassium channel current by 33.27% (15nM)-63.6% (45nM), confirming the sensitivity of the test system.

In conclusion, HSK3486 showed no evidence of potassium channel blockage, with an IC_{50} value of > 16.15 μM based on the detected postperfusion working solution concentrations.

2.3.2 Effect of HSK3486 on Action Potentials in Rabbit Purkinje Fiber

The potential effect of HSK3486 (6, 20, or 60 μM) and propofol (30, 100, or 300 μM) on action potentials was assessed in rabbit cardiac Purkinje fibers at frequency cycle lengths of 2000, 1000, and 600 ms (non-GLP Study No. PCL-0038). Results showed that both HSK3486 and propofol had no significant effect on resting membrane potential, action potential amplitude, and maximum rate of depolarization. Both HSK3486 and propofol were shown to shorten action potential duration in rabbit cardiac Purkinje fibers, with the effect of HSK3486 being much less than propofol. The shortening of action potential duration in Phase II may be caused by HSK3486's blocking of the L-type calcium channel.

2.3.3 Effect of HSK3486 on Cardiovascular Assessment in Beagle dogs

The effect of HSK1346 on cardiovascular safety was investigated in 4 studies in Beagle dogs (Study No. SPD13-026, 13-0461SP, D13-S082-SP, and D13-S082-2SP). Mild and transient changes in the ECG and blood pressure (as well as respiration and body temperature as described

in the IB) were observed after HSK3486 drug injection. These returned to normal when the animals regained consciousness, and can be viewed as an extension of the pharmacological effects of common to anesthetics drugs on target cells, rather than any special toxicity or side effects.

In Study No. SPD13-026, the cardiovascular effects and respiratory effects, were examined in 4 male Beagle dogs given a single IV dose of HSK3486 (1.2, 2.5, or 5 mg/kg) in comparison to propofol medium and long chain fat emulsion injection (6 mg/kg) using a Latin square crossover design. Prior to dose administration, each animal had a vascular access port surgically implanted to allow direct measurement of arterial blood pressures. Short periods of tachycardia (< 2 minutes) and hypertension (≤ 30 seconds; mean arterial pressures > 180 mmHg) occurred at 5 mg/kg. HSK3486 produced less effect on sinus arrhythmia and pulse pressure, suggesting less effect on autonomic tone compared to propofol.

In the GLP Study No. 13-0461SP, the potential effect of HSK3486 on the cardiovascular system was assessed in Beagle dogs (4 males and 4 females, implanted with telemetry devices) given an IV injection of HSK3486 at 0 (normal saline), 1, 2, or 4 mg/kg, in a double Latin square design. Respiratory rate, blood pressure and ECG were continually monitored from 3 to 5.5 hours pre-dose to 8 hours post-dose. When anesthesia was given, statistically significant transient changes in some parameters of ECG and blood pressure, respiration, and body temperature occurred compared with normal saline. Heart rates (HRs) at 2 and 4 mg/kg were accelerated in a dose-dependent manner, various blood pressure indexes were lowered, and the corresponding RR interval was shortened. No statistically significant changes were observed at other time points and there were no trended changes. QTcF was prolonged at all dose levels in a dose-dependent manner. The maximum prolongation was 17.45%. No trended changes were observed in ECG parameters (QRS voltage, $T_{p\text{-}e}$ interval, and respiratory rate). Results of separate and combined analysis of male and female animals were consistent; thus, there was no gender specificity. In conclusion, HSK3486 showed no adverse effect on the cardiovascular or respiratory systems in conscious dogs. The temporary changes in cardiovascular and respiratory functions that accompanied treatment-induced short-term anesthesia were not seen when animals recovered.

In the non-GLP Study No. D13-S082-SP, Beagle dogs (2 males and 2 females, prepared for telemetry) were given IV administrations of HSK3486 (2 mg/kg), propofol medium and long chain fat emulsion injection (10 mg/kg), or vehicle control (normal saline) in a Latin crossover design with at least one day between doses. Evaluated parameters (ECG, blood pressure) were continuously monitored from about 3.5 hours pre-dose to about 6 hours post-dose. Blood samples were collected from animals treated with HSK3486 or propofol pre-dose and up to 3 hours post-dose. At the same time points, no trended changes were observed for the HSK3486, propofol, and vehicle control groups in ECG parameters, QRS voltage, and $T_{p\text{-}e}$ interval.

Statistically significant transient changes in ECG, blood pressure, respiration, and body temperature occurred with HSK3486 or propofol compared with the vehicle control (Table 2.1). No trend of changes occurred in any parameter at 1 to 3 hours post-dose for HSK3486 or propofol compared with the vehicle control. In conclusion, HSK3486 showed a significantly lower effect on the cardiovascular system as compared with propofol.

Table 2.1 Maximum changes in cardiovascular parameters (%) and their corresponding time points

	HSK3486		Propofol	
Parameter	**Δ%**	**Time points**	**Δ%**	**Time points**
Heart Rate	28.39% ↑	10 min	53.08% ↑	2 min
RR Interval	31.96 ↓	2 min	29.22 ↓	2 min
PR Interval	17.37 ↓	2 min	20.71 ↓	2 min
QRS Duration	11.09% ↑	10 min	10.87% ↑	20 min
QT Interval	↔	NA	↔	NA
QTcF	13.49% ↑	20 min	11.29% ↑	20 min
ST Segment	194.44% ↓	20 min	228.57 ↓	10 min
Blood Pressure, Systolic	12.96% ↓	20 min	20.14% ↓	20 min
Blood Pressure, Diastolic	8.52% ↓	20 min	15.97% ↓	5 min
Mean Arterial Pressure	10.50% ↓	20 min	16.48% ↓	10 min
Pulse Pressure Difference	19.89% ↓	20 min	32.04% ↓	20 min
Body Temperature	2.47% ↓	30 min	1.82% ↓	30 min

↓ = decrease; ↑ = increase; NA = not applicable.
Δ% = [(b1-b0) - (a1-a0)]/a1 × 100, where b1 denotes the value at HSK3486/propofol post-dose time point, b0 denotes value at HSK3486/propofol pre-dose time point, a1 denotes corresponding value at normal saline post-dose time point, and a0 denotes value at normal saline pre-dose time point.
Source: Study No. D13-S082-SP

In the non-GLP Study No. D13-S082-2SP, the potential effect of HSK3486 on the cardiovascular and respiratory systems was assessed using different IV dosing rates and in comparison with propofol. Beagle dogs (2 males and 2 females, prepared for telemetry) were given a single IV dose of HSK3486 at 2 mg/kg, or an injection of propofol medium and long chain fat emulsion at 10 mg/kg, in a cross experiment design with at least 1 day washout period. Injection rates were 2 mg/kg/15s and 10 mg/kg/15s (rate 1), 2 mg/kg/60s and 10 mg/kg/60s (rate 2), or 2 mg/kg/120s and 10 mg/kg/120s (rate 3) for the 2 groups, respectively. Evaluated parameters included ECG, blood pressure, and respiratory function from at least 2 hours pre-dose to approximately 4 hours post-dose. Compared with pre-dose data, treatment with HSK3486 showed an increased trend in HR, reaching the highest level at 2 minutes post-dose and returning to normal at 2 hours post-dose. An increased amplitude of HR was seen, and RR and PR intervals were shortened correspondingly. The QRS duration was shortened and the QTcF

interval was slightly prolonged from 5 to 15 minute post-dose and returned to normal at 30 minutes post-dose. Compared with pre-dose data, blood pressure parameters were decreased within 20 minutes post-dose. Compared with propofol, HSK3486 showed the increased amplitude of HR was larger within 15 minutes post-dose using rates 1 and 2 (fast infusion), and was smaller using rate 3 (slow infusion). The HSK3486 effect of decreased blood pressure was less than propofol. Comparison of the different rates in administration of HSK3486 showed that the increased amplitude of HR was larger using rates 1 and 2 (fast infusion) and smaller using rate 3 (slow infusion). The decreased amplitude of blood pressure was smaller using rates 1 and 2 (fast infusion), and larger using rate 3 (slow infusion). All these differences were mild/minor. In conclusion, neither HSK3486 nor propofol showed an adverse effect on the cardiovascular or respiratory systems in conscious dogs. Treatment-induced short-term anesthesia with both HSK3486 and propofol produced transient effects on HR, QRS, and QTcF intervals, blood pressure, and respiratory rate. Minor differences were seen for HSK3486 and propofol in parameter changes and when the compounds were used at different IV administration rates.

2.4 Summary of Clinical Experience

As of August 5, 2023, 30 Phase I–III clinical studies have been initiated or planned for HSK3486; 27 have been completed and 3 are ongoing or planned. In China, the planned indications include gastrointestinal endoscopy (Marketed), induction of general anesthesia (Marketed), fiberoptic bronchoscopy (Marketed), induction and maintenance of general anesthesia (Marketed), and Sedation during mechanical ventilation during intensive care (Marketed). Sedation and anesthesia in gynecological outpatient surgery (Marketed). In the US and EU, the currently planned indication is induction of general anesthesia.

Among the 27 completed studies, a total of 1370 participants were exposed to HSK3486, including 286 healthy participants, 16 participants with hepatic impairment, 20 participants with renal impairment, 236 participants who underwent colonoscopy, 15 participants who underwent gastroscopy, 324 participants who underwent induction of general anesthesia for elective surgery, 135 participants undergoing fiberoptic bronchoscopy, 122 participants who underwent induction and maintenance of general anesthesia for elective surgery, 126 participants undergoing mechanical ventilation in ICU, and 90 participants undergoing gynecology outpatient surgery.

Results showed that HSK3486 featured quick onset, rapid regaining of consciousness, and had approximately 4-5 times the potency of propofol. The types of drug-related adverse events caused by HSK3486 were consistent with those of propofol. Cardiovascular-related adverse events including hypotension, bradycardia, and prolonged QTc interval caused by HSK3486 were comparable to those caused by propofol. However, incidences of injection site pain and respiratory-related adverse events (respiratory depression, apnea, and hypoxia), and the

proportion of patients requiring assisted ventilation were lower with HSK3486 than propofol. Notably, HSK3486 evoked no/less injection site pain in comparison to propofol.

2.4.1 Pharmacokinetics and Metabolism in Humans

Phase I PK studies showed that HSK3486 was widely distributed after a single IV administration, the plasma exposure level increased approximately proportionally to the dose, and the clearance did not change in a dose-dependent manner.

After a single IV dose of HSK3486 given in 1 minute to healthy adult Chinese/Australian participants at 0.128-0.9 mg/kg, exposure level and peak plasma concentration increased approximately in a dose-proportional manner, C_{max} exposure range was 2046 ± 509 ng/mL to 13904 ± 3570 ng/mL, $AUC_{0\text{-}inf}$ exposure range was 6360 ± 1273 ng·min/mL to 49564 ± 7278 ng·min/mL, but the clearance was in a range of 16-27 mL/min/kg for all dose groups, showing no dose-dependence. The plasma concentration of HSK3486 was characterized by three-phase elimination, with corresponding half-lives of 0.537 minutes (0.11-1.34 minutes) ($t_{1/2}$, α), 6.26 minutes (3.97-10.2 minutes) ($t_{1/2}$, β), and 105 minutes (38.3-279 minutes ($t_{1/2}$, γ), similar to propofol.

For healthy Australian participants with continuous infusion (0.288 mg/kg bolus + 1 mg/kg/h infusion; 0.540 mg/kg bolus + 2 mg/kg/h infusion), the concentration of HSK3486 after a 1-minute bolus injection fell rapidly to the level comparable to that after a 30-minute IV infusion, and rapidly achieved a near steady-state level, with the average plasma “apparent steady state” levels at 900 and 1400ng/mL, respectively. When the dose increased by 2 times, mean C_{max} and $AUC_{0\text{-}inf}$ both increased by approximately 1.6 times.

The CYP2B6 inducer, rifampicin, had no significant effect on the PK profiles of HSK3486. At clinical doses, HSK3486 had no inductive effect on human CYP1A2, CYP2B6, and CYP3A4 and basically no inhibitory effect on human CYP1A2, 2A6, 2C8, 2C9, 2D6, 2E1, and 3A4.

2.4.2 Clinical Cardiac Safety

A pooled retrospective cardiodynamic evaluation of 5 studies (HSK3486-101, HSK3486-109, HSK3486-202, HSK3486 SAD_02, and HSK3486 SAD_03) was performed to evaluate the effects of HSK3486 on QTc and other ECG parameters. Three of these studies evaluated the effects of single doses of HSK3486 (or active comparator propofol) in healthy participants, while HSK3486-202 evaluated a bolus of HSK3486 or propofol in patients undergoing general anesthesia prior to surgery, and HSK3486 SAD_03 evaluated the effects of a bolus of HSK3486 followed by a 30 minute continuous infusion in healthy participants. Twelve-lead ECGs were collected in each study at pre-specified time points prior to dosing and post-dose at up to 24 hours. The primary ECG objective was to evaluate the effect of HSK3486 on the QTc interval

corrected for HR using the Fridericia method (QTcF) using a concentration-QTc analysis (primary analysis).

HSK3486-101 and pooled HSK3486-101, HSK3486-109, HSK3486 SAD_02, and HSK3486 SAD_03 studies were selected for the concentration-QTc analyses. Since study HSK3486-202 was conducted in patients rather than healthy participants, data from this trial were not included in the pooled concentration-QTc analysis.

HSK3486 had a clinically relevant effect on HR (increase) across all studies, with similar effects also observed for participants on propofol. In HSK3486-109, the highest heart rate increases with both HSK3486 and propofol were observed at 1 minute post-dose, ranging up to 14.6 bpm for HSK3486 0.6 and 0.4 mg/kg boluses, up to 18.4 bpm for propofol 3.0 and 2.0 mg/kg boluses, and up to 26.7 bpm for a HSK3486 0.540 mg/kg bolus followed by a 30 minute continuous infusion at 2 mg/kg/h. The heart rate subsequently decreased across dose groups and, by 8 minutes to 24 hours post-dose, ranged from -5.6 to 4.8 bpm on HSK3486 treatments and -7.2 to 6.3 bpm on propofol treatments. These effects on heart rate are as expected for anesthetic agents – a rapid increase in heart rate immediately following a bolus (due to the compensatory response to immediate vasodilatation and drop in blood pressure) followed by a slow decline in heart rate due to reduced sympathetic tone from sedation.

HSK3486 demonstrated uncertain effects on QTc, with inconsistent evidence of a QTc increase immediately after bolus dosing (simultaneous with the large, rapid heart rate increase). This QTc increase was generally similar to the by-time point effects observed for propofol; it is difficult to determine whether this represents a true effect on cardiac repolarization due to the concomitant changes in heart rate and autonomic tone. QTc/concentration hysteresis was present. In the pooled analysis of studies HSK3486-101, HSK3486-109, HSK3486 SAD_02, and HSK3486 SAD_03, the concentration-QTc model demonstrated a positive slope for the relationship between HSK3486 plasma concentration and ΔQTcF. The concentration-QTc model predicted effects were highest for the HSK3486 bolus of 0.540 mg/kg plus 2 mg/kg/h infusion (mean ΔQTcF 14.2 ms; 90% UCI 18.5 ms; geometric mean C_{max} 8314 ng/mL) and for the HSK3486 bolus of 0.810 mg/kg (mean ΔQTcF 13.8 ms; 90% UCI 17.9 ms; geometric mean C_{max} 7947 ng/mL). The model predicted that the 90% UCI for ΔQTcF would be crossed at a C_{max} of 3230 ng/mL.

The by-time point analyses for all 5 trials generally demonstrated a peak QTcF increase of 10-15 ms nearly immediately following a bolus of either HSK3486 or propofol. In general, the magnitude of QTc increase was similar for both HSK3486 and propofol. The largest QTcF increase observed was 25.5 ms for the HSK3486 0.540 mg/kg bolus plus 2 mg/kg/h infusion cohort. There were signs of a dose dependence in 3 of the 5 studies (HSK3486-202, HSK3486 SAD_02, and HSK3486 SAD_03), but not in HSK3486-101, HSK3486-109. The joint plots of

plasma concentration and ΔQTcF over time showed that the time course of QTc increase did not match the time course of plasma concentration (hysteresis). In the 4 trials that included single boluses of HSK3486, plasma concentrations of HSK3486 fell very rapidly after the completion of the bolus, while ΔQTcF also rose very rapidly but did not consistently decline in parallel with HSK3486 plasma concentration, often remaining elevated for up to several hours after the bolus.

The reasons for the discordance between the time course of HSK3684 plasma concentration and ΔQTcF are unclear. This discordance may have been related to the small cohort sizes and the timing of ECG and PK collections in each trial, QT/RR hysteresis related to the rapid change in heart rate, or changes in autonomic tone and hemodynamics following bolus infusions of potent anesthetics.

3 Study Rationale

3.1 Selection of the Clinical Dose, Negative, and Positive Control Treatments

Regulatory guidance (ICH E14) has emphasized the need to obtain clear robust data on the effect of new chemical entities on ECG parameters with focus on cardiac repolarization as measured by the QTc duration. Though many clinical studies may be conducted during a drug development program, they usually have insufficient sample size, infrequent sampling of ECG data, or the use of inadequate controls to overcome the high rate of spontaneous change in QTc duration.

A Thorough QT/QTc study, as described in the ICH E14 guidance, is generally performed using a supratherapeutic dose of a drug to assess the ECG effects of a supratherapeutic exposure (C_{max}) in order to be able to inform the ECG effects of the drug not only at the mean therapeutic exposure expected in typical participants, but also for subsets of participants who, due to intrinsic or extrinsic factors, might be expected to have an exposure substantially larger than the mean exposure at the maximum clinical dose. However, due to HSK3486's sedative properties, it is not feasible to dose healthy participants with a dose that achieves supratherapeutic exposure, as this would likely produce significant respiratory depression requiring intubation and mechanical ventilation, which would make QT assessments uninterpretable.

This study will be performed in healthy participants to eliminate variables known to have an effect on ECG parameters (concomitant drugs, diseases, etc.). The maximum clinical dose of HSK3486 is expected to be a 0.4 mg/kg bolus, with the option for a 0.2 mg/kg 'top off' dose. The use of a second, smaller dose does not increase C_{max}.

The proposed study will therefore utilize a 6-sequence, randomized, double-blind (except moxifloxacin), placebo -controlled single dose crossover design and will evaluate the effects of a single 0.4 mg/kg IV bolus of HSK3486 on QTc and other ECG parameters.

Since significant hysteresis was observed in the concentration-QTc model used in the prior pooled analysis, this study will utilize the by-time point analysis as the primary analysis.

In consideration of the large heart rate effect observed in the previous trials, the individual QT correction method (QTcI) will be used as the primary QT correct method.

3.2 Sample Size

Approximately 48 participants will be randomized, with the expectation that at least 42 evaluable participants will have data from all treatment periods. Moxifloxacin will be included as a positive control to validate the sensitivity of the assay to detect small increases from baseline QTcF duration (Florian et al)[1]. The sample size power calculations are detailed in Section 5.1.

3.3 Objectives and Endpoints of the Study

3.3.1 Primary Objective

- To assess the effects of HSK3486 single dose on cardiac repolarization (QTc interval of the electrocardiogram).

3.3.2 Secondary Objectives

- To assess the effects of a single dose of HSK3486 on other ECG parameters relative to a moxifloxacin positive control and to placebo.
- To demonstrate assay sensitivity of the study using oral moxifloxacin 400 mg single dose as a positive control.
- To evaluate the safety and tolerability of a single dose of HSK3486 in healthy adult participants.

3.3.3 Primary Endpoint

- Change-from-baseline in QTc, corrected for HR using the individual QT correction method (QTcI) – ΔQTcI, which will be used as the dependent variable for calculation of model-derived ΔΔQTcI for the by-time point analysis (primary analysis).

3.3.4 Secondary Endpoints

- Change-from-baseline heart rate (HR), QTcF, PR, and QRS intervals, which will be used as the dependent variable for calculation of model-derived ΔΔHR, ΔΔQTcF, ΔΔPR, and ΔΔQRS for the by-time point analysis, respectively
- Categorical outliers for QTcI, QTcF, HR, PR, and QRS intervals
- Treatment-emergent changes in ECG morphology

4 Study Design

This is a single dose, placebo and positive controlled, 3-period, 6-sequence crossover design study to evaluate the effects of a single dose of HSK3486 50 mg (predicted to cover therapeutic exposures at the clinical dose for induction of anesthesia) on the QTc interval and other ECG parameters. Evaluation will be performed in a blinded manner (except for moxifloxacin, which will not be blinded) in approximately 48 healthy participants with 8 participants randomized to each sequence. Each period will consist of 1 day of treatment followed by a 5-7 day washout period. Participants will be randomized to 1 of 3 treatment groups in each period using the Williams design with 8 participants assigned to each sequence and with C representing active treatment (HSK3486), B, the positive control (moxifloxacin), and A, the control (placebo) treatment.

4.1 Treatment Groups

Approximately 48 healthy adult participants will be randomized. Dropouts will not be replaced. Enrollment may be increased to allow for at least 42 evaluable participants.

The 3 treatments will be:

Treatment ID	Treatment Description
A	Placebo (Negative Control) single IV bolus
B	Moxifloxacin (Positive Control) 400 mg single oral dose
C	HSK3486 0.4 mg/kg IV bolus

Participants randomized to a treatment sequence will receive a single dose of the assigned study treatment on the morning of Day 1in each period. The placebo and HSK3486 will be identical in appearance volume infused. Moxifloxacin will not be blinded. All treatments will be administered in the fasted state, followed by a washout of 5-7 days.

The random order design of treatment used the following Williams design with 8 participants being assigned to each sequence:

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

	Period 1	Period 2	Period 3
Sequence 6	B	A	C

Continuous cardiac 12-lead ECG recordings for ECG extraction will be collected for 1 hour prior to and through 24 hours after dosing on Day 1 of each study period.

4.2 Study Drug Administration

Within each period, all doses will be administered in the morning on Day 1, following an overnight fast of at least 8 hours. Participants will be restricted from water consumption 1 hour before and 4 hours after dosing. A standardized lunch will be provided after the 4 hours post-dose ECG extraction window. Participants will undergo continuous oxygen saturation monitoring to avoid potential hypoxemia due to respiratory depression.

4.3 Selection of Participants

4.3.1 Inclusion Criteria

Participants eligible for enrollment must meet all inclusion criteria and none of the exclusion criteria:

1. Is capable of understanding the informed consent and is willing and able to provide written informed consent.
2. Is willing to comply with all protocol procedures.
3. Healthy, male participants from18 through 45 years of age, inclusive, at Screening and healthy, female participants of child-bearing and non-childbearing potential from 18 to 45 years of age, inclusive, at Screening.
4. Female patients are eligible if they are of reproductive potential and have a negative serum pregnancy test (beta human chorionic gonadotropin), are not breastfeeding, and do not plan to become pregnant during the study and agree to use 2 highly effective birth control methods during the study OR if they are not of child bearing potential (i.e., surgically [bilateral oophorectomy, hysterectomy, tubal ligation, tubal occlusion or bilateral salpingectomy] or naturally sterile [amenorrhea for 12 months without an alternative medical reason with confirmatory follicle-stimulating hormone level of $\geq$ 40 mIU/mL. The amenorrhea should not be induced by a medical condition such as anorexia nervosa, hypothyroid disease or polycystic ovarian disease, or by extreme exercise. It should not be due to concomitant medications that may have induced the amenorrhea such as oral contraceptives, hormones, gonadotropin-releasing hormones, anti-estrogens, or selective estrogen receptor modulators]). Surgical sterilization to have occurred a minimum of 6 weeks, or at the investigator's discretion, prior to screening.

5. Body weight >50 kg, body mass index between 18–30 kg/m^2, inclusive.
6. LVEF ≥ 65%
7. Male participants with partners of childbearing potential must use a male barrier method of contraception (i.e., male condom with spermicide) in addition to a second method of acceptable contraception from check-in until 90 days after discharge or abstain from sexual intercourse from the first dose of study drug until 90 days after discharge. Acceptable methods of contraception for female partners include: hormonal injection, combined oral contraceptive pill or progestin/progestogen-only pill, combined hormonal patch, combined hormonal vaginal ring, surgical method (bilateral tubal ligation or Essure® [hysteroscopic bilateral tubal occlusion]), hormonal implant, hormonal or non-hormonal intrauterine device, over-the-counter sponge with spermicide, cervical cap with spermicide, diaphragm with spermicide.

   An acceptable second method of contraception for male participants is vasectomy that has been performed at least 90 days prior to the screening visit, with verbal confirmation of surgical success.

   For male participants (even with a history of vasectomy), sexual intercourse with female partners who are pregnant or breastfeeding should be avoided unless condoms are used from the time of the first dose until 90 days after discharge. Male participants are required to refrain from donation of sperm from check-in until 90 days after discharge.
8. A male who has been vasectomized less than 3 months prior to Screening must follow the same procedure as a non-vasectomized male. Males must agree to not donate sperm 90 days after the last dose of study drug.
9. Willing to abstain from alcohol for at least 48 hours prior to Day -1 and through the End of Study Visit.
10. Willing to abstain from caffeine-containing products for at least 48 hours prior to Day -1 and through the End-of-Study Visit.

4.3.2 Exclusion Criteria

Participants meeting any of the following exclusion criteria at Screening and check-in will not be enrolled in the study:

1. Past or present clinically significant systemic disease as judged by the Investigator including, but not limited to, clinically significant medical abnormalities such as psychiatric, neurologic, pulmonary, respiratory, cardiac, gastrointestinal, genitourinary, renal, hepatic, metabolic, endocrinologic, hematological, or autoimmune disorders.
2. History of significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance, unless approved by the investigator (or designee).
3. Clinically significant infection/injury/illness within 1 month prior to Day -1.

4. Current or past history of seizure disorder, including alcohol- or stimulant-related seizure, febrile seizure, or significant family history of idiopathic seizure disorder.
5. Positive test results for HIV-1/HIV-2 Antibodies, Hepatitis B surface Antigen (HBsAg), or Hepatitis C Antibody (HCVAb).
6. Pregnant or lactating females at Screening or Day -1.
7. Knowledge of any kind of cardiovascular disorder/condition/procedure known to increase the possibility of QT prolongation or history of additional risk factors for torsade de pointes (e.g., heart failure, hypokalemia, hypomagnesemia, congenital Long QT syndrome, or family history of Long QT Syndrome, or Brugada Syndrome), or cardiac conduction disorders.
8. Clinically significant laboratory abnormalities that would place the participant at undue risk in the Investigator's opinion, including, but not limited to, serum alanine aminotransferase (ALT) or serum aspartate aminotransferase (AST) > 2 × ULN (the upper limit of the reference range at Screening or Day -1) or direct bilirubin greater than the upper limit of reference range at Screening (congenital nonhemolytic hyperbilirubinemia [e.g., suspicion of Gilbert's syndrome based on total and direct bilirubin] is not acceptable) or elevated creatine kinase (CK) at screening or check-in (one repeat test allowed). Laboratory assessments may be repeated once at the discretion of the Investigator.
9. Resting supine systolic blood pressure greater than 140 mmHg; resting supine diastolic blood pressure greater than 90 mmHg at Screening or Day -1. Blood pressure measurements may be repeated once at the discretion of the Investigator.
10. Resting supine heart rate less than 50 beats per minute or greater than 100 beats per minute at Screening or Day -1 (may be repeated once at the discretion of the Investigator). Minor deviations are acceptable if considered to be of no clinical significance by the Investigator.
11. Oxygen saturation (SpO2) below 95% at Day -1.
12. Abnormal 12-lead ECG at Screening or Day -1 (a single repeat is allowed), including:
    a. QTcF > 450 ms
    b. QRS > 110 ms
    c. PR > 200 ms
    d. Second or third-degree AV block
13. Any rhythm other than sinus rhythm, which is interpreted by the Investigator to be clinically significant at Screening or Day -1.
14. Febrile illness with temperature ≥38.0°C for >7 days before Screening or Day -1.
15. eGFR < 90 mL/min (estimated using Cockroft-Gault equation)
16. Pre-existing condition interfering with normal gastrointestinal anatomy or motility, hepatic and/or renal function that could interfere with the absorption, metabolism, and/or

excretion of study drug (e.g., history of bariatric surgery or intestinal bypass surgery; uncomplicated appendectomy and hernia repair is allowed). Cholecystectomy is not allowed.

17. Participation in another clinical study of an investigational drug (or medical device) within the last 30 days (or 5 half-lives, whichever is longer) prior to dosing.
18. Donation of blood from 3 months prior to screening, plasma from 2 weeks prior to screening, platelets from 6 weeks prior to screening.
19. Family history of unexplainable sudden death at <50 years of age.
20. History of unexplained loss of consciousness, unexplained syncope, unexplained irregular heartbeats or palpitations, clinically significant head injury or near drowning with hospital admission.
21. Use or intend to use any medications/products known to alter drug absorption, metabolism, or elimination processes, including St. John's wort, within 30 days prior to dosing, unless deemed acceptable by the investigator (or designee).
22. Use or intend to use any prescription medications/products within 14 days prior to dosing, unless deemed acceptable by the investigator (or designee); except for oral contraceptives.
23. Use or intend to use slow-release medications/products considered to still be active within 14 days prior to check-in, unless deemed acceptable by the investigator (or designee).
24. Use or intend to use any nonprescription medications/products including vitamins, minerals within 7 days, and phytotherapeutic/herbal/plant-derived preparations within 21 days prior to check-in, unless deemed acceptable by the investigator (or designee).
25. Known allergic reactions to moxifloxacin or any study medication or history of tendonitis or tendon rupture as a result of moxifloxacin or any other quinolone type drug use.
26. Consumption of caffeine (including caffeine-containing beverages and/or food) or xanthine within 48 hours of study treatment or excessive caffeine consumption defined as ≥ 8 cups of coffee or equivalent (where a cup is 8 oz and has 100 mg of caffeine per cup, i.e., 800 mg per day) within 7 days prior to Screening.
27. Thyroid stimulating hormone (TSH) < LLN or TSH > 6 mIU/L at Screening or Day -1. TSH may be repeated once at the discretion of the Investigator.
28. Current or recent (< 6 months from Screening) hepatobiliary disease.
29. History of regular alcohol consumption exceeding 10 drinks/week for females or 14 drinks/week for men (1 drink = 5 oz of wine or 12 oz of beer or 1.5 oz of hard liquor) within 6 months prior to Screening.
30. History of drug or alcohol abuse within 2 years prior to Day -1 as defined by the Diagnostic and Statistical Manual of Mental Disorders 5th Edition.

31. Performing or unwilling to refrain from strenuous physical activity, which could cause muscle aches or injury, including contact sports, at any time from 3 days prior to Day 1 through the End-of-Study Visit.
32. Positive urine drug screen at screening or positive alcohol breath test result or positive urine drug screen at Day -1.
33. Prisoners or participants who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical disease (e.g., infectious disease) must not be enrolled.
34. Any condition or situation that, in the opinion of the Investigator, would prevent proper evaluation of the safety or efficacy of the study drug according to the study protocol (e.g., poorly compliant participant, poor venous access, allergies to medical plastics/latex).
35. Administration of a coronavirus disease 2019 (COVID-19) vaccine in the past 30 days prior to dosing (Day 1).
36. Positive COVID-19 test at Day -1.
37. Have previously completed or withdrawn from this study or any other study investigating HSK3486.
38. Ingestion of poppy seed-, Seville orange-, or grapefruit-containing foods or beverages within 7 days prior to check-in.
39. Receipt of blood products within 2 months prior to check-in.

4.4 ECG Methodology

4.4.1 12-Lead ECG Acquisition

ECGs will be obtained digitally using a M12R continuous 12-lead ECG recorder (Manlius, NY, USA) or comparable system, which will collect ECGs on Day -1 of the first treatment period and on Day 1 of each treatment period. ECGs to be used in the analysis will be selected at predetermined time points as detailed below and will be read centrally using a high-resolution manual on-screen caliber semiautomatic method with annotations. On Day -1 of the first treatment period, the recording will run for approximately 24 hours; these recordings will be used for derivation of QTcI. On Day 1 of each ECG collection period, the recording will be started approximately 1 hour prior to the nominal dosing time through approximately 24 hours post dosing in order to obtain the ECGs as detailed below.

ECGs will be obtained as replicate (up to 10) 12-lead ECGs within each extraction window at each time point. Replicate 12-lead ECGs will be extracted at the following times in each period of the study:

Day 1:

- -60, -45, and -30 minutes pre-dose

- 1, 3, 5, 8, 10, 15, 30, and 60 minutes after dosing
- 2, 3, 4, 6, 10, and 24 hours after dosing

4.4.2 Pharmacokinetic Sampling

Blood for PK sampling will be obtained from all participants on Day 1 of each treatment period. Samples will be analyzed for plasma concentrations of HSK3486. Since only plasma levels for HSK3486 will be of interest, only samples from participants receiving HSK3486 will be analyzed. The time points on Day 1 that will be used for such sampling will be:

pre-dose, end of dose; 1, 2, 5, 10, 30, and 60 minutes following dosing; 2, 3, and 6 hours following dosing.

The ECG extractions will take place before the actual plasma sampling to avoid changes in autonomic tone from bloodletting.

4.5 ECG Analysis Methods

ECGs will be sent to the core cardiac ECG laboratory for a treatment-blinded high-resolution measurement of the cardiac intervals and morphological assessment by a central cardiologist blinded to the study treatment.

4.5.1 TQT Plus ECG Extraction Technique

The core laboratory will use TQT Plus, an advanced computer-assisted and statistical process utilized to extract ECGs from continuous 24-hour recordings collected in TQT studies. During protocol-specified ECG extraction windows, 10-second digital 12-lead ECG tracings will be extracted from continuous recordings. The TQT Plus method enables the extraction of a high-quality data set by identifying periods of recordings with the lowest available heart rate variability and noise.

The ECGs will be extracted according to the following principles:

- The actual times of dosing, extraction windows, and PK sampling will be communicated to the central ECG laboratory by the study center personnel.
- The TQT Plus process identified periods of stable heart rate on the continuous 12-lead ECG tracing within the 5-minute extraction window. Stability will be defined as variation in the heart rate and other ECG parameters from beat-to-beat lower than a predefined threshold. If the TQT Plus method results in a low number of consecutive, readable cardiac cycles in the 5-minute time point, the time point will be fully reviewed manually.
- Replicate, non-overlapping 14-second ECGs will be extracted in close succession within each extraction window.

4.5.2 Early Precision QT Analysis

Twelve-lead ECGs will be extracted in up to 10 replicates from each nominal time point prespecified in the protocol. The median value of each parameter from the set of evaluable beats in each extracted replicate will be calculated, and then the mean of all available medians from the nominal time point will be used as the participant'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 a cardiac safety specialist. All low confidence beats will be reviewed manually by a 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 for assessment of ECG morphology and, 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 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. A cardiologist will review the caliper placements and perform the morphology evaluation of each replicate. A replicate will only be reported if it has 3 approved, usable beats.

4.5.3 Cardiodynamic ECG Assessment

A continuous 12-lead ECG and Holter data acquisition will be in place for 25 hours on Day 1 in each treatment period, as detailed in Section 4.4.1.

The following ECG parameters will be measured and calculated: Heart rate (HR), RR, PR, QRS, QT, QTcI, and QTcF. ECG morphology will also be assessed.

The primary analysis will be based on the by-time point analysis to evaluate the effect of HSK3486 on the placebo-corrected change-from-baseline QTcI (ΔΔQTcI) at each post-dosing time point using the Intersection Union Test (IUT). The intent is to exclude an effect on ΔΔQTcI with the upper bound of 2-sided 90% confidence interval (CI) ≥ 10 ms at any post-dose time point.

In addition, the effect of HSK3486 on ΔΔHR, ΔΔQTcF, ΔΔPR, and ΔΔQRS will be evaluated at each post-dosing time point ('by-time point' analysis).

An analysis of categorical outliers will be performed for changes in heart rate (HR), PR, QRS, QTcI, and QTcF. Morphological analysis will also be performed.

Assay sensitivity will be evaluated by using the by-time point analysis of the effect of moxifloxacin on ΔΔQTcI using a similar model as for the primary analysis, as detailed in Section 6.2.

4.6 Safety ECGs and ECG Discontinuation Criteria

Standard digital 12-lead safety ECGs will be recorded at screening, check-in, and pre-dose and 3 hours post-dose on Day 1 of each treatment period in order to detect any immediate ECG effects on participant safety. ECGs will be repeated at discharge. Safety ECGs will be recorded and evaluated by the site Investigator or Sub-Investigator.

Any on-therapy safety ECG with a QTcF > 500 ms (as defined by automatically measured intervals) at the 3-hour time point or at any other time point where a safety ECG will be recorded by the site, will be confirmed by a second ECG taken within 1 hour. If the second repeat safety ECG will be confirmed, to have a QTcF > 500 ms, the participant will be discontinued from the study.

4.7 Study Conduct

The Phase 1 unit will ensure that the same meal timing and components as well as activity levels and general conditions will be as close as possible in each treatment period of this study. Participants will be supine and resting comfortably for at least 10 minutes before each of the time points for ECG and PK samples.

5 Statistical Analysis Plan

5.1 Sample Size Determination

A sample size of 48 participants was chosen to obtain 42 evaluable participants who complete the study. Assuming a 1-sided 5% significance level and a within-participant SD of 8 ms for ΔQTcI for all treatment groups and a true mean difference of 3 ms in ΔQTcI between HSK3486 and placebo, based on the calculation of the sample size for a TQT study (Zhang and Machado)[2], a sample size of 42 evaluable participants who complete the study will provide a power of 88% to demonstrate that the upper bound of all the 2-sided 90% CIs on ΔΔQTcI will fall below 10 ms for up to 14 post-dose time points.

5.1.1 Sample Size Determination for Assay Sensitivity

Based on the calculation of the sample size for a TQT study (Zhang and Machado)[2] with multiplicity controlled by using a Hochberg procedure (Hochberg)[3], as the test is performed at four time points separately (1, 2, 3, and 4 hours), a 1-sided 5% significance level (with adjusted 1-sided significance levels of 5%, 2.5%, 1.67%, and 1.25%) is used along with a within-participant SD of 8 ms for ΔQTc and a true effect of moxifloxacin of 10 ms, a sample size of 42 evaluable participants with moxifloxacin and 42 evaluable participants with placebo will provide a power of 98% to demonstrate assay sensitivity of excluding a mean difference of 5 ms in ΔQTcI between moxifloxacin and placebo groups, i.e., the lower bound of the 2-sided 90% CI of least squares mean of ΔΔQTcI will exceed 5 ms at least one of the 4 pre-specified time points.

5.2 Participant-Level Analysis Populations and Record-Level Analysis Sets

The participant-level analysis populations for cardiodynamic ECG assessment are defined in Table 5.1.

Table 5.1 Participant-level analysis populations for cardiodynamic ECG assessment

Population	Participants
QT/QTc analysis set	All participants who received at least 1 dose of study treatment (HSK3486, moxifloxacin, or placebo) and had centrally reviewed cardiodynamic ECG measurements at baseline as well as on-treatment with at least 1 post-dose time point with a valid change-from-baseline value for at least 1 ECG parameter. The QT/QTc analysis set will be used for the by-time point, categorical, and morphological analyses of the cardiodynamic ECG parameters.

The observations that will be included at a record/time point level in the statistical analyses described in this SAP are defined in Table 5.2.

Table 5.2 Record-level analysis sets for cardiodynamic ECG assessment

Analysis Sets	Time Points
By-time point analysis set	Each post-baseline time point that has a change-from-baseline observation. This analysis set is defined for all continuous ECG parameters for participants in the QT/QTc analysis set.
Categorical and morphological analysis set	For continuous ECG parameters, each post-dose time point that has a 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. This analysis set is defined for all participants in the QT/QTc analysis set.

5.3 General Methodology

All statistical analyses will be performed using the statistical software SAS for Windows Version 9.4 or higher (SAS Institute, Inc., Cary, NC). Data collected from all randomized participants will be presented in data listings. Both absolute values and change-from-baseline values for each participant will be provided where applicable. All continuous data will be listed with the same precision as presented in the database. Data listings will be sorted by participant ID, treatment, visit/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, QTcI, and QTcF), data will be summarized including number of participants (n), mean, median, standard deviation (SD), 2-sided 90% confidence interval (CI; based on t-distribution if not otherwise stated), 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. 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 tenth decimal place.

Unused/unscheduled ECG data and safety assessment data will not be included in ECG analysis tables, but will be included in participant data listings, if available.

Data from participants excluded from an analysis population will be presented in the data listings but will not be included in the calculation of summary statistics.

During the analysis and reporting process, any deviations from the statistical plan designed for this protocol will be described and justified in the final report.

5.4 Baseline

For all continuous ECG parameters, baseline will be the average of the derived ECG intervals from the 3 ECG time points prior to dosing (-60, -45, and -30 minutes) on Day 1 within each respective period. For ECG morphologic analysis, baseline includes findings observed in any of the replicates from the 3 ECG time points prior to dosing on Day 1 within each respective period.

5.5 Concept and Criteria for ECG Analysis

The ECG interval durations will be participated to the following plan: to describe central tendency and outlier effects for each of HR, PR, QRS, QT, QTcI, and QTcF. New ECG morphology and changes will also be evaluated. “New” is defined as “not present on any baseline ECG but present on any on-treatment ECG”.

5.6 QT Correction Methods

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

The Fridericia’s correction (QTcF), which will be used as the secondary QT correction method, is defined as QTcF (ms) = QT (ms) /$[RR(ms)/1000]^{1/3}$.

The primary QT correction method will be the optimized HR-corrected QT interval, QTcI. QTcI will be derived from the HRs by using all QT/RR data from the full 24-hour Holter recording (all acceptable beats) on Day -1 of Period 1. Based on QT/RR pairs from each participant, QTcI correction coefficient will be derived from a linear regression model: log(QT) = log(a) + b×log(RR'). The coefficient of log(RR’) for each participant, bi, will then be used to calculate QTcI for each participant as follows: $QTcI_i = QT/(RR')^{bi}$.

RR′ will be derived using the unadjusted RR, 1-minute average RR, 2-minutes average RR, 1-minute weighted average RR and 2-minutes weighted average RR, respectively. Hysteresis adjustment will also be applied to the extracted ECGs with the same duration of averaging, and weighted averaging. The weighted average (exponential decay, based on an AR(1) filter) details are described as below.

- Using AR(1) filter as $w_i = (1-\lambda)\frac{\lambda^i}{1-\lambda^{N+1}}$, where N is the number of beats within the preceding one or three minutes with λ being calculated as $\lambda = 0.05^{\frac{1}{L+1}}$ (L being 60 * length of the adjustment window in minutes).
- Using these values, the weighted average is computed as $RR' = \frac{\sum_{i=1}^{N} w_i \times RR_i}{N}$

The individual correction coefficients, bi, will be listed and summarized in a table using arithmetic mean, standard error (SE), number of participants, and 90% CI (based on t-distribution).

5.7 Optimized QTcI Baseline Review

The continuous ECG recording for visit Period 1 Day -1 will be reviewed for QT and RR measurements in all recorded beats by the iCOMPAS software. All beats determined to be "low confidence" beats were reviewed manually by a Clario ECG analyst and adjudicated using pass-fail criteria. Low confidence beats will be chosen based on absolute values (upper and lower bounds) and relative thresholds (percent change from beat to beat) in QT and RR values that were defined and listed in the internal data analysis plan maintained by Clario.

The low confidence beats found to be acceptable by manual review and the beats categorized as high confidence by the iCOMPAS software will be included in the beat-to-beat analysis.

5.8 Evaluation of QT-RR Correction Methods

The relationship between QTc (QTcF and QTcI [optimized] using unadjusted RR, 1-minute average RR, 2-minutes average RR, 1-minute weighted average RR and 2-minutes weighted average RR) and RR interval will be investigated using on-treatment data (HSK3486, moxifloxacin, and placebo) by a linear regression model: QTc = c+ d x RR. Mean QTc and RR values from all nominal post-baseline time points will be used. The RR coefficient for each participant, d_i, will then be used to calculate the mean of squared slopes (MSS) for each of the different QT-RR correction methods. The average on treatment slope closest to zero (the smallest MSS) for HSK3486 and placebo will be used to evaluate the appropriate heart rate correction methods.

For evaluation of the HR-corrected QT interval, a scatter plot of QTc and RR intervals by treatment (HSK3486, moxifloxacin, and placebo) in different colors will be generated with a simple linear regression line fit to all QTc-RR pairs across all participants and treatments. Decile plots of QTc and RR intervals by treatment with a mean fitted regression line (90% CI) from a linear mixed-effects model for each treatment will also be created for each QT correction method.

6 Analysis

6.1 By-Time Point Analysis (Primary Analysis)

A by-time point analysis of HSK3486 effects on HR, PR, QRS, and QTc (QTcI and QTcF) will be performed, with the by-time point analysis for QTcI as the primary analysis.

The ECG analysis will be based on defining the central tendency of all ECG interval parameter changes (HR, QTcI, QTcF, PR, and QRS) as a change-from-baseline. The baseline ECG data will be compared to the post-dose ECGs.

To support the by-time point statistical modeling described below, descriptive statistics (e.g., number of participants, mean, SD, median, maximum, and minimum) will be used to summarize the absolute values of the ECG variables and the corresponding change-from-baseline values at each time point by treatment group. Data-based (i.e., not model-based) 2-sided 90% CI descriptive statistics will be summarized for the change-from-baseline data only.

Besides the above analysis using descriptive statistics, a by-time point analysis will also be performed using statistical modeling as follows.

The by-time point analysis for QTcI will be based on a linear mixed-effects model with change-from-baseline QTcI (ΔQTcI) as the dependent variable, period, sequence, time (i.e., post-dose time points on Day 1: categorical), treatment (HSK3486, moxifloxacin, and placebo), and time-by-treatment interaction as fixed effects, and baseline QTcI as a covariate. An unstructured covariance matrix will be specified for the repeated measures at post-dose time points for participant within treatment period. The model will also include a participant-specific random effect on the intercept. If the model with both a participant-specific random effect and an unstructured covariance matrix at post-dose time points for participant within treatment period fails to converge, the participant-specific random effect will be dropped firstly and other covariance matrices such as compound symmetry will be considered, in this order, until the model converges. From this analysis, the LS mean, SE, and 2-sided 90 % CI of ΔΔQTcI will be calculated for the contrast “HSK3486 versus placebo” and the contrast “moxifloxacin versus placebo” at each post-dose time point on Day 1. If the upper bound of the 2-sided 90% CI of LS mean ΔΔQTcI is below 10 ms for all post-dose time points, HSK3486 will be concluded not to have a significant effect on QT interval prolongation.

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

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

Where ECG = QT/QTc analysis set, SUBJID = participant number, TREAT = treatment (HSK3486, moxifloxacin, and placebo), TIME = nominal post-dose time point, BASE = baseline QTcF, PERIOD = period, SEQUENCE = sequence, and DQTc = ΔQTcI.

6.2 Assay Sensitivity (Secondary Analysis)

The analysis to show assay sensitivity will be based on ΔQTcI interval of moxifloxacin. The same model will be used as described for the primary analysis (by-time point analysis). For the time points 1, 2, 3, and 4 hours after dose administration, the contrast in treatment ΔΔQTcI = "moxifloxacin – placebo" will be tested against the 1-sided null hypothesis ΔΔQTcI ≤ 5 ms at the 5% significance level. Multiplicity will be controlled using the Hochberg procedure (Hochberg)[3]. Therefore, contrast estimates will be rearranged to test the 1-sided null hypothesis ΔΔQTcI ≤ 5 ms. The time point with the largest *P* value for the rearranged contrast will be tested against the 1-sided significance level of 0.05. If the largest *P* value is significant (i.e., less than 0.05), assay sensitivity will be considered shown. Otherwise, the second largest *P* value will be tested against the 1-sided significance level of 0.025. If this *P* value is significant (i.e., less than 0.025), assay sensitivity will be considered shown. Otherwise, the third largest *P* value will be tested against the 1-sided significance level of 0.0167. If this *P* value is significant (i.e., less than 0.0167), assay sensitivity will be considered shown. Otherwise, the smallest *P* value will be tested against the 1-sided significance level of 0.0125. If this *P* value is significant (i.e., less than 0.0125), assay sensitivity will be considered shown. Otherwise, assay sensitivity will not have been demonstrated among the 4 selected time points. In addition, 2-sided 90% CIs will be obtained for ΔΔQTcI = "moxifloxacin – placebo" at all time points and used in the figures.

6.3 Categorical Outlier Analysis (Secondary Analysis)

Results for categorical outliers will be summarized in frequency tables with counts and percentages for both number of participants and number of time points. Participant data will be summarized using the count of distinct participants that fall into the category and the percentage of the total number of participants. 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 participants or number of time points) for each treatment group will be used as the denominator in the calculation of percentages unless otherwise specified.

A participant or time point will be determined as an outlier if the following criteria (which are assessed separately) are met for the ECG intervals (Table 6.1).

Table 6.1 Criteria for determining a participant or time point categorical outlier

ECG interval	Categorical outlier criteria
QTc (QTcI in 5 ways if calculated and 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 > 100 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 group on the basis of incidence rates. A participant will be counted only once for a particular outlier event if the participant 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 participants within a treatment group.

6.4 Morphological Analysis (Secondary Analysis)

Morphological analyses will be performed based on the ECG waveform interpretation determined by the central ECG laboratory's cardiologist. Changes from baseline to the post-dose ECGs will be evaluated for each treatment group.

All findings will be presented in the ECG listings. New onset findings will be presented as the percentage of participants meeting the "new" criteria ("new" means an ECG finding that is not present on any baseline ECG [that is, any ECG recorded prior to receipt of the first dose of study drug] and becomes present on at least 1 on-treatment ECG during that treatment period) for the following variables.

- Atrial fibrillation
- Atrial flutter
- Non-sustained ventricular tachycardia
- Mobitz I second degree atrioventricular (AV) block (Wenckebach)
- Mobitz II second degree AV block
- 2:1 AV block
- Third degree heart block (i.e., complete heart block)
- Complete right bundle branch block
- Complete left bundle branch block
- ST segment elevation
- ST segment depression
- T-wave inversion
- Myocardial infarction
- New abnormal U waves

6.5 Deviations in Analysis from Statistical Plan and Other Issues

During the analysis and reporting process, any deviations from the statistical plan designed for this protocol will be described and justified in the final report.

7 References

1. Florian JA et. al. Population pharmacokinetic and concentration-QTc models for moxifloxacin: pooled analysis of 20 Thorough QT studies. J Clin Pharmacol 2011;51:1152-1162.
2. Zhang, Joanne and Machado, Stella G. Statistical Issues Including Design and Sample Size Calculation in Thorough QT/QTc Studies. Journal of Biopharmaceutical Statistics. 2008;18:3, 451-467.
3. Hochberg Y. A sharper Bonferroni procedure for multiple tests of significance. Biometrika. 1988; 75, 800–802.

8 Tables, Figures, and Listings

8.1 Tables

Number	Title	Comments
14.3.10.1.1	QTcI individual correction coefficients with descriptive statistics	Number of participants (n), mean, SD, median, minimum, and maximum will be given (Section 5.6).
14.3.10.1.2	MSS for different QT-RR correction methods	MSS for HSK3486, moxifloxacin and placebo will be given (Section 5.6).
14.3.10.2	Baseline values of ECG parameters with descriptive statistics	Number of participants (n), mean, SD, 90% CI, median, minimum, and maximum from descriptive analysis will be given by treatment for each ECG parameter (Section 5.4).
14.3.10.3.1-14.3.10.3.9	Observed values of QTcI in 5 ways if calculated, QTcF, HR, PR and QRS with descriptive statistics	n, mean, SD, 90% CI, median, minimum, and maximum from descriptive statistics will be given by treatment and post-dose time point (Section 6.1).
14.3.10.4.1-14.3.10.4.9	Change-from-baseline QTcI in 5 ways if calculated, QTcF, HR, PR, and QRS (ΔQTcI, ΔQTcF, ΔHR, ΔPR, and ΔQRS) at each time point with descriptive analysis	n, mean, SD, 90% CI, median, minimum, and maximum from descriptive analysis will be given by treatment and post-dose time point for each ECG parameter (Section 6.1).
14.3.10.5.1-14.3.10.5.9	Change-from-baseline QTcI in 5 ways if calculated, QTcF, HR, PR, and QRS (ΔQTcI, ΔQTcF, ΔHR, ΔPR, and ΔQRS) at each time point with statistical modeling	n, LS mean, SE, and 90% CI from the statistical modeling will be given by treatment and time point (Section 6.1).

Number	Title	Comments
14.3.10.6.1-14.3.10.6.9	Placebo-corrected change-from-baseline QTcI in 5 ways if calculated, QTcF, HR, PR, and QRS (ΔΔQTcI, ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS) at each time point with statistical modeling	n, LS mean, SE, and 90% CI from the statistical modeling will be given by treatment and time point (Section 6.1).
14.3.10.7	Placebo-corrected change-from-baseline QTcI (ΔΔQTcI) for assay sensitivity test	LS mean, lower bound of 2-sided 90% CI, *P* value, and adjusted alpha will be given at each of the 4 prespecified time points (Section 6.2).
14.3.10.8	QTc outliers per absolute category	Number (%) of participants and time points with QTc > 450 and ≤ 480 ms, > 480 and ≤ 500 ms, or > 500 ms by treatment (Section 6.2).
14.3.10.9	QTc outliers per change-from-baseline category	Number (%) of participants and time points with ΔQTc > 30 and ≤ 60 ms, or > 60 ms by treatment (Section 6.2).
14.3.10.10	Categorical analyses for HR, PR, and QRS	Number and percentage (%) of participants and time points with ΔPR/baseline > 25% and PR > 200 ms at post-dose; ΔQRS/baseline > 25% and QRS > 120 ms at post-dose; ΔHR/baseline < -25% and HR < 50 bpm at post-dose; and ΔHR/baseline > 25% and HR > 100 bpm at post-dose (Section 6.2).
14.3.10.11	Morphological analysis (new onset by finding)	Number (%) of participants and time points in each finding will be given by treatment group (Section 6.4).
14.3.10.12	Numbers of participants and number of observations of participant- and record-level analysis sets	Number of participants and number of observations available will be provided for participant- and record-level analysis sets, respectively (Section 5.2).

8.2 Figures

Number	Title	Comments
14.3.10.22.1-14.3.10.22.9	Observed QTcI in 5 ways if calculated, QTcF, HR, PR and QRS across time points with descriptive analysis	Mean and 90% CI from descriptive analysis will be given by treatment for each ECG parameter (Section 6.1).
14.3.10.23.1-14.3.10.23.9	Change-from-baseline QTcI in 5 ways if calculated, QTcF, HR, PR, and QRS (ΔQTcI, ΔQTcF, ΔHR, ΔPR, and ΔQRS) across time point with descriptive analysis	Mean and 90% CI from descriptive analysis will be shown by treatment for each ECG parameter (Section 6.1).
14.3.10.24.1-14.3.10.24.9	Change-from-baseline QTcI in 5 ways if calculated, QTcF, HR, PR, and QRS (ΔQTcI, ΔQTcF, ΔHR, ΔPR, and ΔQRS) across time point with statistical modeling	LS mean and 90% CI from the statistical modeling will be shown by treatment (Section 6.1).
14.3.10.25.1-14.3.10.25.9	Placebo-corrected change-from-baseline QTcI in 5 ways if calculated, QTcF, HR, PR, and QRS (ΔΔQTcI, ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS) across time point with statistical modeling	Mean and 90% CI from descriptive analysis will be shown by treatment for each ECG parameter (Section 6.1).
14.3.10.26.1	Scatter plot of QTc versus RR by treatment	Scatter plots of QTc and RR intervals by treatment with regression lines will be given (Section 5.8).
14.3.10.26.2	QTc-RR decile plot by treatment	QTc-RR decile plots with linear mixed-effects line and 90% CI will be given (Section 5.8).

8.3 Listings

Number	Title	Comments
16.2.9.1.1-16.2.9.1.9	QTcI in 5 ways if calculated, QTcF, HR, PR, and QRS intervals - observed and change-from-baseline values as well as categorical outliers	Sections 6.1 and 6.3.
16.2.9.2	Morphological analysis	Section 6.4.

9 Approvals

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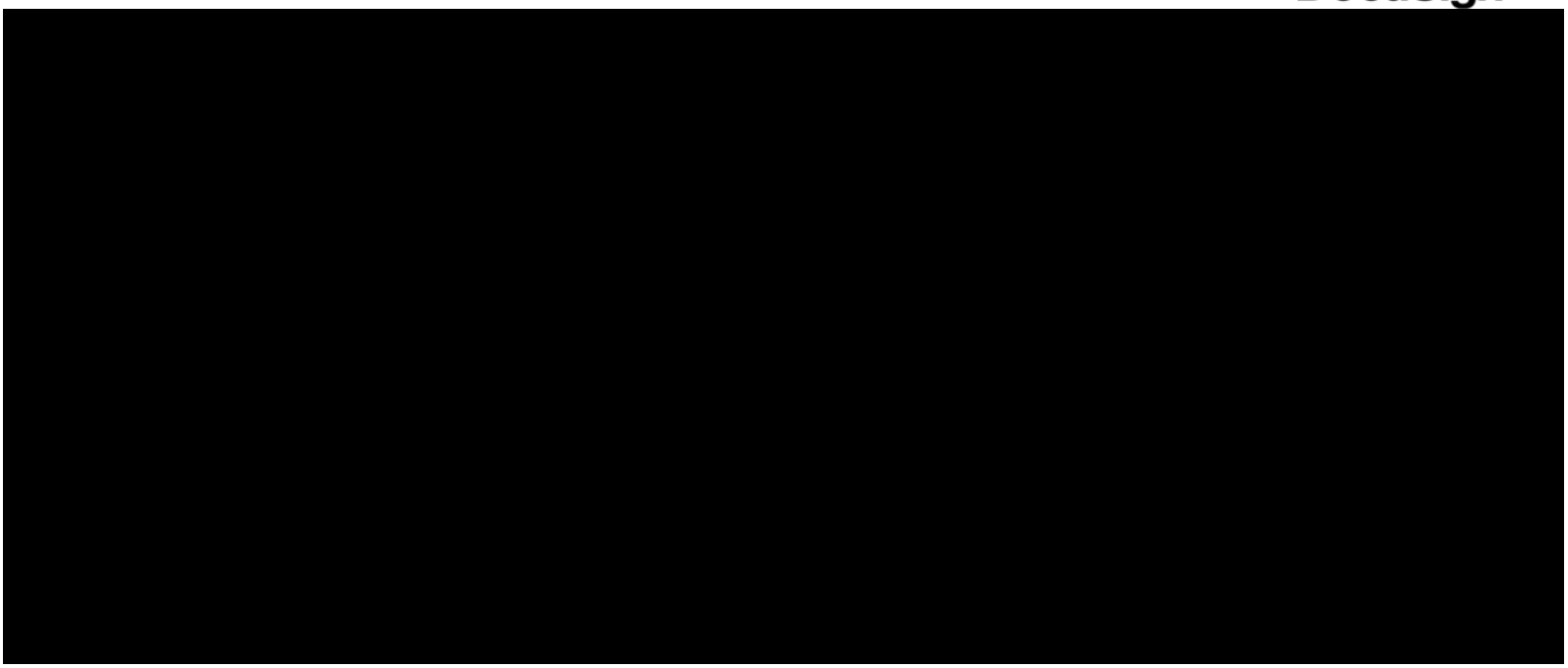

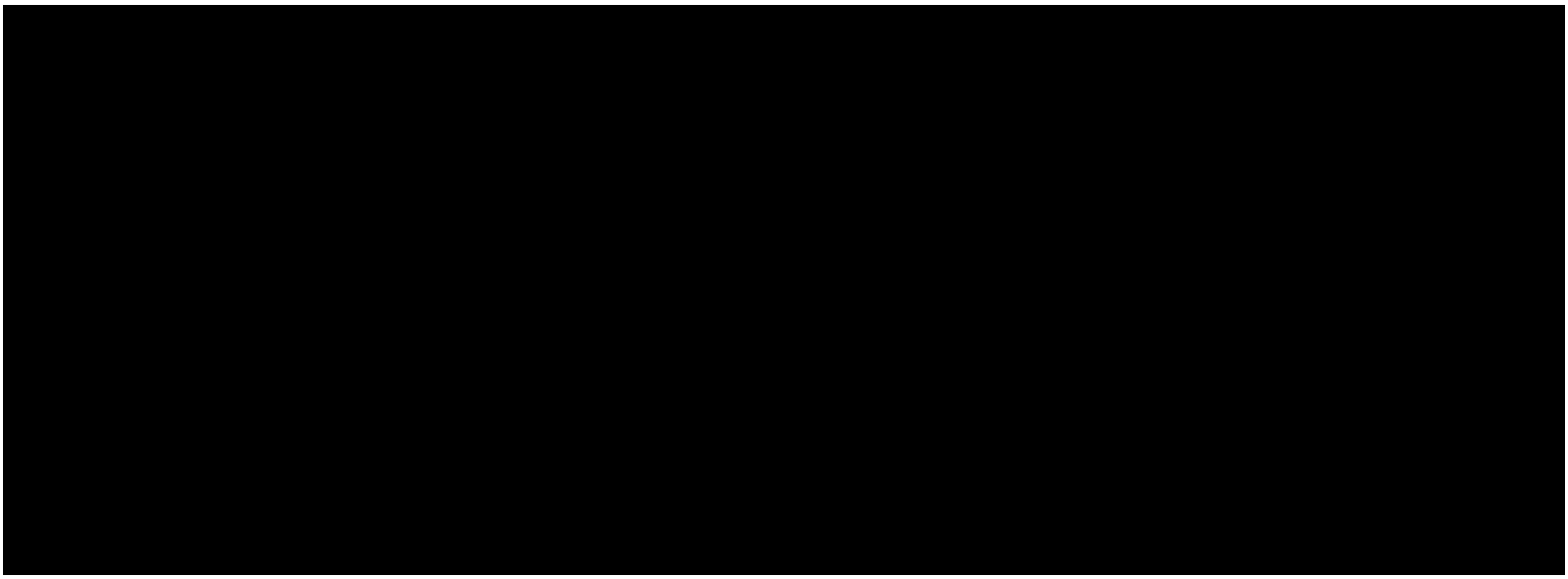

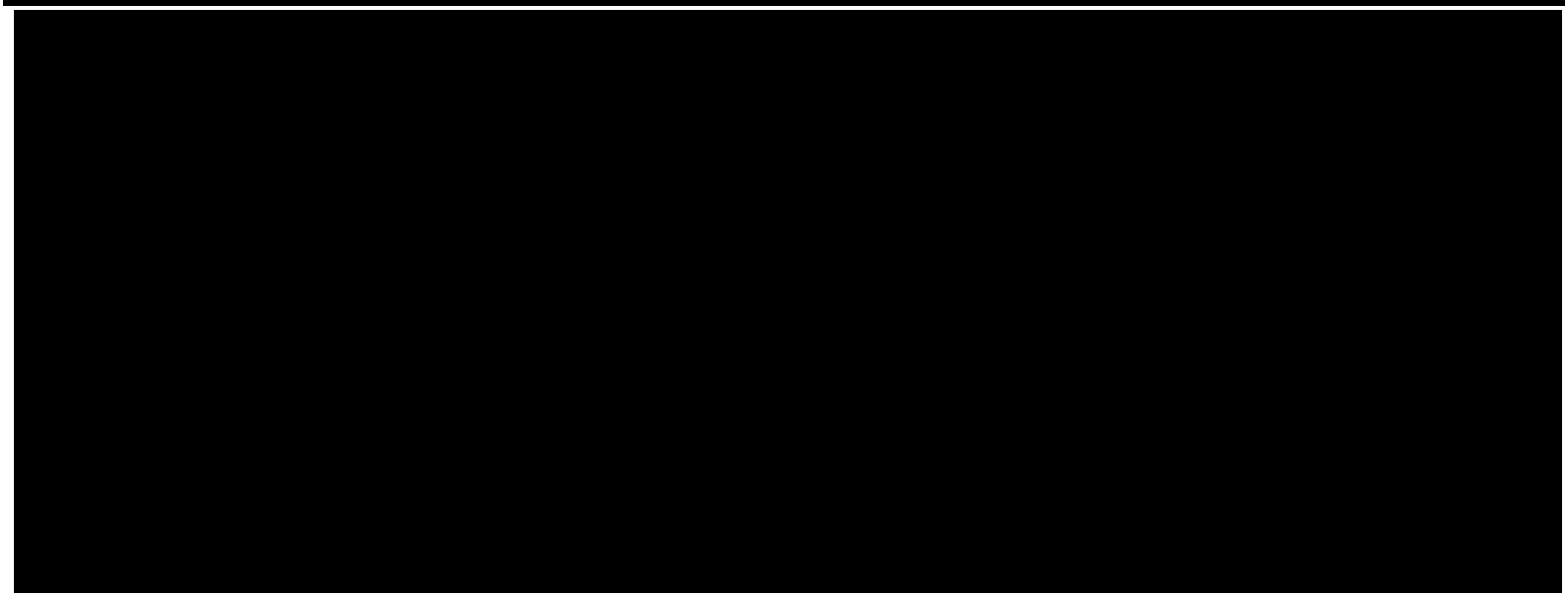

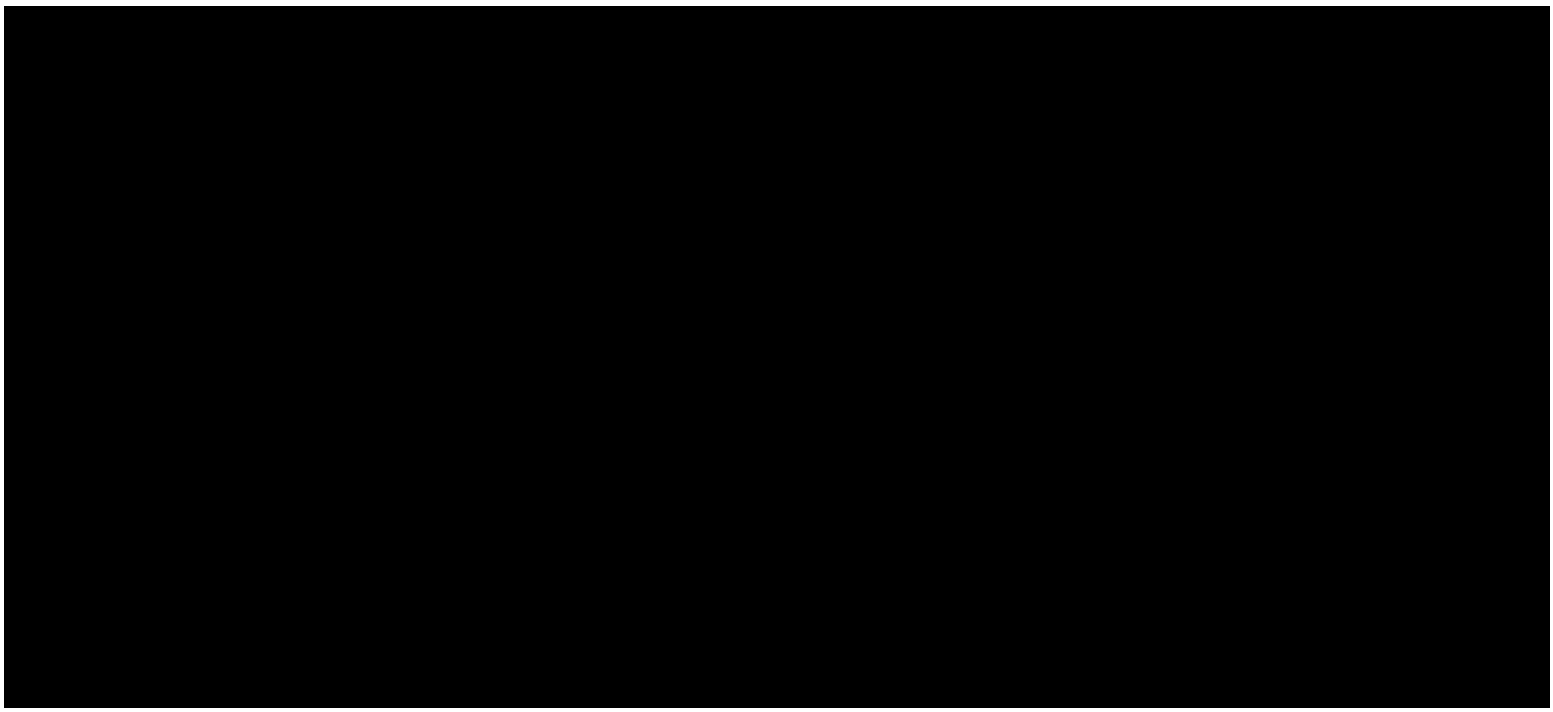

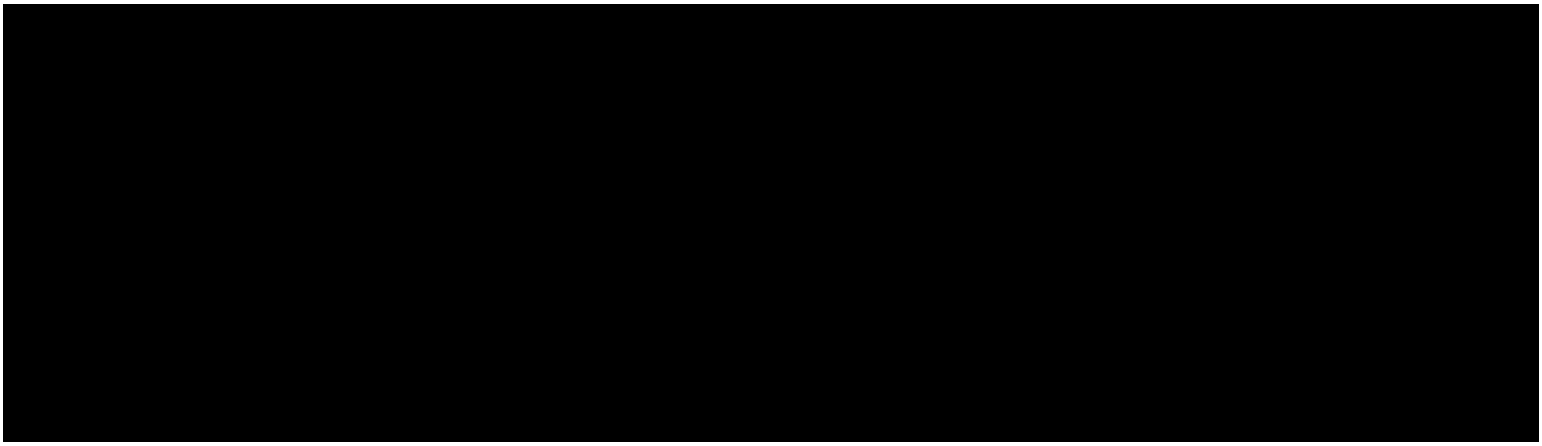

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