Cover page

Protocol Number:	HSK3486-112 , V3.0
	Document date: 15 March 2024
Protocol Title:	A Randomized, Positive and Placebo-Controlled Study to Evaluate the Effects of HSK3486 Administration on Cardiac Repolarization in Healthy Subjects
NCT Number:	NCT06795204

TITLE PAGE

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Protocol Number:	HSK3486-112
Version Number:	V3.0
Study drug:	HSK3486
Sponsor:	Xizang Haisco Pharmaceutical Co., Ltd.
Legally Registered Address:	Happy Homeland Economic Development Zone, Jieba Township, Naidong District, Shannan City, Tibet Autonomous Region
Version Date:	15 March 2024

SIGNATURE PAGE

We have read this clinical study protocol, with the protocol no.: HSK3486-112, and version no.: V3.0 (Version Date: 15 March 2024), and agree to perform the responsibilities of the sponsor in accordance with the Local laws, Declaration of Helsinki, Good Clinical Practice (GCP) issued by the National Medical Products Administration (NMPA) and this clinical study protocol.

[redacted]

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[redacted]

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[redacted]

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Signature Date

SUMMARY TABLE OF PROTOCOL AMENDMENT CHANGES

Summary Table of Protocol Amendment Changes

File History		
File Name	**Version No.**	**Version Date**
Protocol	V1.0	03 January 2024
Protocol	V2.0	05 March 2024
Protocol	V3.0	15 March 2024

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ABBREVIATIONS LIST

Abbreviation	English name
ADL	Activities of Daily Living
AE	adverse event
ALT	Alanine aminotransferase
APA	Action potential amplitude
AST	Aspartate Transaminase
AUC	area under the curve
BMI	body mass index
bpm	Beats per minute
CI	Confidence interval
CK	creatine kinase
CL	plasma clearance
C_{max}	maximum concentration
CTCAE	common terminology criteria for adverse event
Δ	Change-from-baseline
ΔΔ	Placebo-corrected change-from-baseline
ECG	electrocardiogram
ECGS	ECG Analysis Set
eCRF	electro-case report form
eGFR	Estimated Glomerular Filtration Rate
FDA	food and drugs administration
g	gram
GCP	good clinical practice
GMP	good manufacturing practice
h	hour
hERG	human Ether-à-go-go Related Gene
HR	Heart rate
HIV	human immunodeficiency virus
IB	Investigator Brochure
IC_{50}	the half maximal inhibitory concentration

Abbreviation	English name
ICH	The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICF	Informed Consent Form
ITT	Intention-To-Treat
kg	kilogram
L	liter
LS	Least squares
LVEF	Left ventricular ejection fraction
MDRD	Modification of Diet Inrenal Disease
mg	milligram
min	minute
mIU/L	Million International Units /liter
mL	milliliter
ms	millisecond
NA	Not applicable
NMPA	national medical products administration
PK	pharmacokinetic
PKCS	pharmacokinetic concentration set
PKPS	pharmacokinetic parameter set
PR	The time from the beginning of the P wave to the beginning of the QRS complex
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
RMP	Resting Membrane Potential
s	second
SAE	serious adverse events
SD	Standard deviation

Abbreviation	English name
SE	Standard error
SS	safety analysis set
SoA	schedule of activities
SOC	System Organ Class
SOP	standard operating procedure
SpO_2	oxygen saturation of blood
SUSAR	suspected unexpected seriousadverse reaction
$t_{1/2z}$	half life
TdP	Torsades de pointes
TEAE	treatment-emergent adverse event
T_{max}	time of maximum concentration
TQT	Thorough QT/QTc
TSH	thyroid stimulating hormone
ULN	Upper Limit of Normal
V_{max}	maximal velocity
V_{ss}	volume of distribution at steady-state

1. PROTOCOL SYNOPSIS

1.1. Synopsis

Protocol Title	A Randomized, Positive and Placebo-Controlled Study to Evaluate the Effects of HSK3486 Administration on Cardiac Repolarization in Healthy Subjects
Protocol Number	HSK3486-112
Sponsor	Xizang Haisco Pharmaceutical Co., Ltd.
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 a single IV bolus of HSK3486 for healthy subjects.
Overall Design	This is a single-center, randomized, blinded (except moxifloxacin hydrochloride tablet), placebo and positive controlled study with a 6-sequence, three-period crossover design in healthy subjects. A blinded design is used for administration of HSK3486 and placebo, and an open-label design is used for moxifloxacin hydrochloride tablet.
Investigational Product	**Study Drug:** HSK3486, Generic name: Ciprofol injection, strength: 20 mL: 50 mg/vial; method of administration: 0.4 mg/kg, IV bolus administration, the time of administration is 30 (±5) seconds. **Placebo:** HSK3486 simulator, strength: 20 mL/vial; method of administration: IV bolus administration, the time of administration is 30 (±5) seconds. **Positive Control:**

	Moxifloxacin hydrochloride tablet, strength: 0.4 g/tablet; method of administration: 0.4 g, Oral administration with 240 mL of warm water on an empty stomach.
Study Duration	This clinical study is planned to last approximately 39 days, including a screening period of 19 days, baseline period and observation period of approximately 14 days (Three periods, washout period of 5 to 7 days between periods), and safety follow-up (7(±1) day after the last dose) of 1 day.
Sample Size	Forty eight (48) subjects will be enrolled in this study, with the expectation that at least 42 evaluable subjects will have data from all treatment periods.
Study Population	**Inclusion Criteria:** Subjects are eligible for inclusion in the study when only all of the following inclusion criteria are met: 1. Ability to understand and comply with protocol requirements and is willing voluntarily sign written informed consent form(ICF). 2. Healthy participants at age from 18 to 45 years old (inclusive) at Screening. 3. Male body weight ≥50 kg, female body weight ≥45 kg, with a body mass index BMI of 19~28 kg/m^2 (inclusive). 4. Left Ventricular Ejection Fraction（LVEF）≥50%. **Exclusion Criteria:** Subjects will be excluded from the study if they meet any of the following criteria: 1. Past or present clinically significant systemic disease as judged by the Investigator including, but not limited to psychiatric, neurologic, pulmonary, respiratory, cardiac, gastrointestinal, genitourinary, renal, hepatic, metabolic, endocrinologic, hematological, or autoimmune disorders. 2. History of allergy to egg or egg products, soybean or soy products. 3. History of significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance. History of allergy to HSK3486 or moxifloxacin or its investigational product excipients, or history of specific allergies (asthma, urticaria, eczema, etc.), or history of tendinitis or tendon rupture due to moxifloxacin or any other quinolone drug. 4. Clinically significant infection/injury/disease within 1 month prior to dosing.

	5. Current or recent (< 6 months from screening) hepatobiliary disease. 6. Current or past history of seizure disorder, including alcohol- or stimulant-related seizure, febrile seizure, or significant family history of idiopathic seizure disorder. 7. Family history of sudden death at <50 years of age. 8. History of unexplained loss of consciousness, unexplained syncope, unexplained irregular heartbeats or palpitations, clinically significant head injury. 9. Pre-existing condition interfering with normal gastrointestinal anatomy or motility, hepatic and/or renal function, or conditions that could interfere with the absorption, metabolism, and/or excretion of study drug (e.g., history of bariatric surgery or intestinal bypass surgery; simple uncomplicated appendectomies and hernia repairs are allowed, but cholecystectomy is not allowed). 10. Positive test results for hepatitis B surface antigen, hepatitis C antibody, treponema pallidum antibody, human immunodeficiency virus (HIV) antigen/antibody combination test. 11. Subjects with previous or suspected difficult airway (e.g., modified Mallampti score III-IV, congenital microglossia, mandibular dysplasia), or respiratory insufficiency, history of obstructive pulmonary disease, history of asthma, sleep apnea syndrome; history of failed tracheal intubation; history of bronchospasm requiring treatment within 3 months prior to screening; acute respiratory infection, and with obvious symptoms such as fever, wheezing, nasal congestion or cough within 1 week prior to baseline. 12. Knowledge of any kind of cardiovascular disorder/condition/procedure known to increase the possibility of QT prolongation or history of risk factors for torsade de pointes (e.g., heart failure, hypokalemia, hypomagnesemia, congenital Long QT syndrome, or family history of Long QT Syndrome. 13. Laboratory tests at screening or baseline judged clinically significant by the investigator, including, but not limited to, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 1.2 × upper limit of normal (ULN)(the upper limit of the reference range at screening or baseline), direct bilirubin > ULN (congenital nonhemolytic hyperbilirubinemia [e.g., suspicion of Gilbert's syndrome based on total and direct bilirubin]

	is not acceptable), creatine kinase (CK) > ULN (one repeat test allowed), thyroid stimulating hormone (TSH) outside normal range (0.75 to 5.6 mIU/L) , serum potassium outside normal range (3.5 to 5.3 mmol/L). 14. Rest sitting vital sign results abnormal and clinically significant at screening or baseline, ear temperature outside normal range, diastolic blood pressure ≥ 90 mmHg or systolic blood pressure ≥ 140 mmHg, heart rate (HR) < 55 beats/min or > 100 beats/min (test can be repeated once according to investigator's judgment). 15. Oxygen saturation (SpO_2) below 95% at baseline. 16. Abnormal 12-lead ECG at screening or baseline (any test abnormality), including any of the following: a) QTcF > 450 ms b) QRS > 110 ms c) PR > 200 ms d) Second or third-degree AV block e) Any rhythm other than sinus rhythm of clinical significance. 17. Estimated Glomerular Filtration Rate (eGFR) < 90 mL/min (estimated using MDRD equation). 18. Participation in another clinical study of an investigational drug (or medical device) within 3 months (or 5 half-lives, whichever is longer) prior to dosing, or previous participation in any other clinical trial related to HSK3486. 19. Donation of blood within 3 months prior to screening, plasma within 2 weeks prior to screening, platelets within 6 weeks prior to screening, or receive blood products within 2 months prior to admission to a the investigational site. 20. Sperm and egg donation program from screening period to 90 days after study end. 21. Pregnant or lactating women or those with positive pregnancy test results. Male or female subjects of childbearing potential do not agree to use an effective method of contraception from the time of signing ICF until 90 days after leaving the investigational site after the last dose (see Appendix 6 for details of specific contraceptive methods). 22. 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 or during the clinical trial.

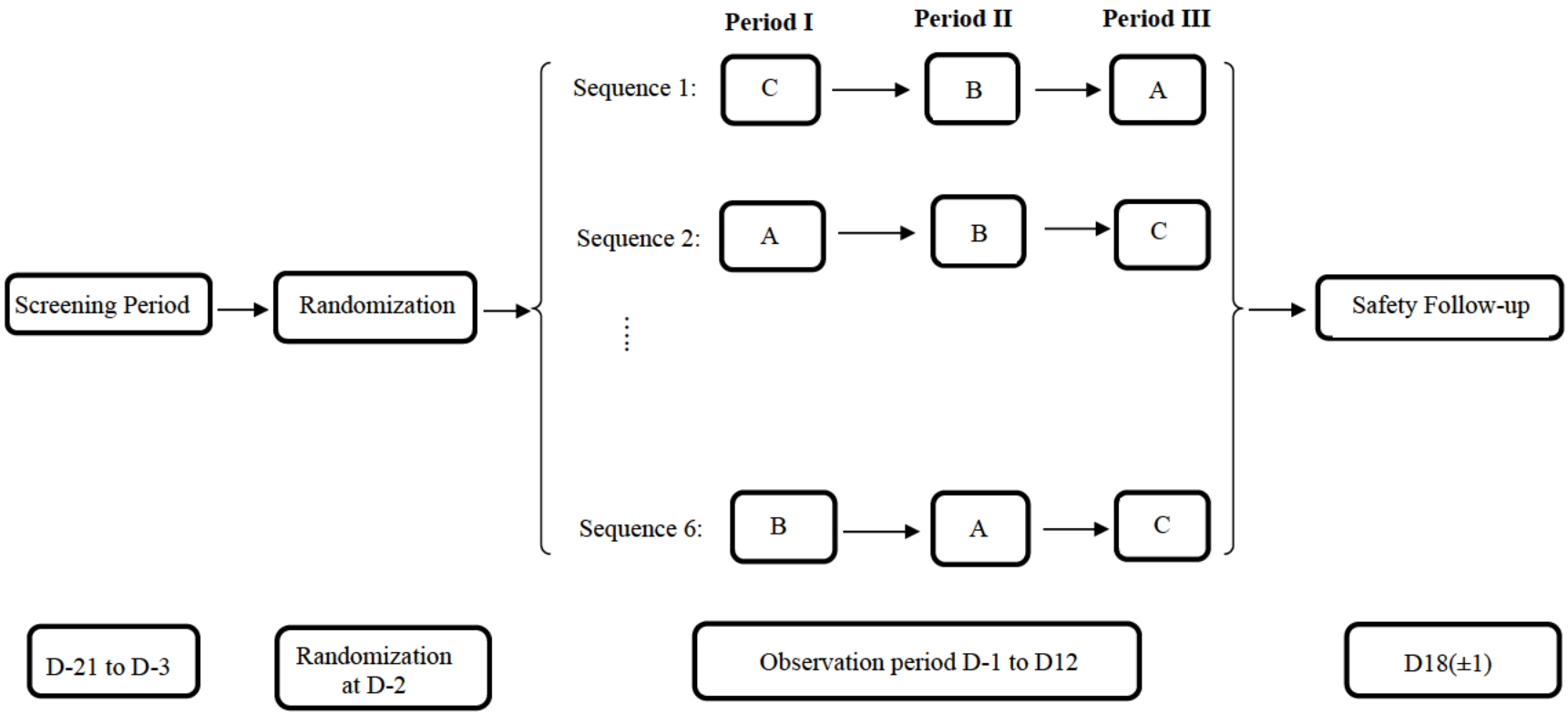
	23. Use or intend to use of any prescription, nonprescription, vitamin, herbal, or nutraceutical within 14 days prior to dosing or during the clinical trial. 24. Smoking (≥ 5 cigarettes per day) within 6 months prior to screening, or inability to quit smoking during the trial. 25. Positive alcohol breath test, or regular drinking within 6 months prior to dosing or during the trial, i.e. drinking more than 21 units (men) or 14 units (women) of alcohol per week (1 unit =360 mL beer or 45 mL spirits or 150 mL wine at 40% alcohol). 26. Positive urine drug abuse screening (morphine, tetrahydrocannabinol, methamphetamine, methylenedioxyamphetamine, ketamine), or history of drug abuse, drug dependence within 6 months prior to screening, or drug use within 3 months prior to screening. 27. Subjects who tested positive for COVID−19 during screening. 28. Eating fruits or foods affecting metabolic enzymes, such as grapefruit (citrus), pomelo, etc., within 7 days prior to screening; and not abstaining from the above beverages, fruits or foods during the study period. 29. Previous chronic excessive consumption (more than 8 cups per day, 1 cup =250 mL) of tea, coffee or caffeinated beverages, or intake of caffeine and/or purine-rich foods or beverages (e.g. coffee, tea, chocolate, caffeinated carbonated beverages, cola, etc.) within 48 hours prior to screening, or refusal to stop drinking tea, coffee and/or caffeinated beverages during the trial. 30. 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 dosing through the end-of-study visit. 31. Subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical disease (e.g., infectious disease). 32. 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 subject, poorly vascular condition, allergies to medical plastics/latex).
Study Procedures	Subjects must sign an ICF in writing on a voluntary basis before participating in the screening process.

	Forty eight (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 3 for dosing sequences), and the study procedure consists of 3 periods, with washout period of 5-7 days between periods. Each dosing sequence contained any of the 3 treatment components: • A: Placebo (HSK3486 simulator) (Negative control); • B: moxifloxacin hydrochloride tablet 0.4g (Positive control); • C: HSK3486 0.4 mg/kg (Study drug). Holter collection, PK blood sample collection and safety examinations shall be completed after the subjects participate in the clinical trial. Holter collection and PK blood sample collection time points are detailed in Section 1.4. Safety examinations: vital signs, physical examinations, 12-lead ECG, clinical laboratory tests (blood routine, urine routine, blood biochemistry (excluding serum electrolytes), coagulation function, thyroid function, serum electrolytes, pregnancy test), adverse events (AE), concomitant drugs and concomitant non-drug therapy observation and recording, etc.
Endpoints	**Primary Endpoint:** The primary endpoint of this clinical trail is the change-from-baseline in QTc interval, corrected for HR using the individual QT correction method (QTcI) – ΔQTcI. **Secondary Endpoints:** Secondary endpoints for this clinical trial include: • 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. • Incidence of AE and serious adverse events (SAE). • Plasma concentration and PK parameters of HSK3486.
Statistical Methods	Detailed statistical analyses are described in the Statistical Analysis Plan. Analysis Population: • **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 Set (SS)** All randomized subjects who received at least one investigational product (HSK3486, moxifloxacin hydrochloride tablet, or placebo) and have post-dose safety evaluation results. • **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. **Cardiac ECG Analysis** **By-Time Point Analysis (Primary Analysis)** A by-timepoint 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. **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). **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. Timepoint data will be summarized using the count of

	timepoints at which the assessments fall into the category and the percentage of the total number of timepoints at which assessments are performed. **Morphological Analysis (Secondary Analysis)** Morphological analysis 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. **Safety Analysis** Safety analysis includes the occurrence of AEs, abnormalities in physical examinations, vital signs, 12-lead ECG and clinical laboratory tests (blood routine, blood biochemistry (excluding serum electrolytes), urine routine, coagulation function, thyroid function and serum electrolytes). Adverse events will be coded using MedDRA and will be summarized by system organ class (SOC) and the preferred term (PT), respectively. The cases, number and incidence of AEs will be calculated according to different investigational products. Descriptive statistics of changes from baseline in laboratory tests, physical examinations, vital signs, and 12-lead ECG for the most severe clinical evaluation results after dosing will be performed in the form of a cross-tabulation pre-and post-dosing (based on normal ranges and/or investigator's judgment of clinical significance). **Pharmacokinetic Analysis** Pharmacokinetic parameter calculations and statistical analyses will be performed using WinNonlin8.3 or later and SAS 9.4 or later. PK parameters of HSK3486 in humans will be calculated using a non-compartmental model.

1.2. Visit Diagram



Note:

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

* The detailed information and complete list of planned treatment sequences are shown in Table 3 Dosing Sequence Listing.

1.3. Schedule of Activities（SoA）

Procedures/ Assessments	Screening Period	Baseline Period	Observation Period													Early Withdrawal[16]	Safety Follow-up
Visit Date	D-21 to D-3	D-2	Period I					Period II					Period III				7(±1) day after the last dose
			D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12		D18(±1)
Informed consent	X																
Demographic information[1]	X																
Medication history/ Medical history/ Surgical history	X																
Smoking history/ Drinking history/ Allergies history	X																
Physical examinations[2]	X	X#													X	X	
Vital signs[3]	X	X	X	X	X			X	X	X			X	X	X	X	
Height and weight (and BMI calculation)[4]	X			X					X					X			
12-lead ECG[5]	X	X	X	X	X			X	X	X			X	X	X	X	
Cardiac ultrasound	X																
Airway assessment[6]	X																
Blood routine[7]	X	X#													X	X	
Urine routine[7]	X	X#													X	X	
Blood biochemistry (excluding serum	X	X#													X	X	

Procedures/ Assessments	Screening Period	Baseline Period	Observation Period												Early Withdrawal[16]	Safety Follow-up
Visit Date	D-21 to D-3	D-2	Period I					Period II					Period III			7(±1) day after the last dose
			D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12		D18(±1)
electrolytes)[7]																	
Coagulation function[7]	X														X	X	
Thyroid function[7]	X														X	X	
Serum electrolytes[8]	X	X#		X					X					X	X	X	
Pregnancy test[9]	X	X#													X	X	
Infectious disease screening	X																
COVID-19 nucleic acid detection[10]	X																
Urine drug abuse screening		X															
Alcohol breath test		X															
Oxygen saturation (pulse)		X															
Inclusion/Exclusion criteria	X	X															
Check-in to the investigational site		X															
Check-in consultation[11]		X															
Assign random number		X															
Dosing				X					X					X			

Procedures/ Assessments	Screening Period	Baseline Period	Observation Period												Early Withdrawal[16]	Safety Follow-up
Visit Date	D-21 to D-3	D-2	Period I					Period II				Period III				7(±1) day after the last dose
			D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12		D18(±1)
ECG monitoring[12]				X					X					X			
Observation of subject's consciousness[13]				X					X					X			
Holter Collection[14]			X	X	X				X	X				X	X	X	
PK blood sample collection[14]				X					X					X		X	
Leaving the investigational site															X	X	
AE record[15]	X----------															----------X	
Concomitant drug[15]	X----------															----------X	
Concomitant non-drug therapy[15]	X----------															----------X	
Telephone follow-up																	X

Explanatory notes:

1. Demographic information include: date of birth (mm yyyy), age, gender, ethnicity, work status, etc.
2. Physical examinations include: skin and mucous membranes, lymph nodes, head and neck, chest, abdomen, skeletal muscle, nervous system, and other parts.
3. Vital signs (including pulse, respiration, ear temperature, blood pressure):
   - Screening Period (D-21 to D-3);
   - Baseline Period (D-2);
   - Period I: D-1, pre-dose (within 1 h) and 2 h (±30 min), 24 h (±1 h) (D2) after the start of post-dose on D1;

- Period II: D5, pre-dose (within 1 h) and 2 h (±30 min), 24 h (±1 h) (D7) after the start of post-dose on D6;
- Period III: D10, pre-dose (within 1 h) and 2 h (±30 min), 24 h (±1 h) (D12) after the start of post-dose on D11;
- or vital signs will be checked upon early withdrawal, and 5-min rest will be required before the examination.

4. Height and weight (and BMI calculation): Height and weight (and BMI calculation) will be performed during screening period (D-21 to D-3), and weight will be measured after emptying urine pre-dose in period I (D1), period II (D6) and period III (D11).
5. 12-lead ECG (including heart rate, PR interval, QRS duration, QT interval, QTcF):
   - Screening Period (D-21 to D-3);
   - Baseline Period (D-2);
   - Period I: D-1, pre-dose (within 2 h) and 2 h (±30 min), 24 h (±1 h) (D2) after the start of post-dose on D1;
   - Period II: D5, pre-dose (within 2 h) and 2 h (±30 min), 24 h (±1 h) (D7) after the start of post-dose on D6;
   - Period III: D10, pre-dose (within 2 h) and 2 h (±30 min), 24 h (±1 h) (D12) after the start of post-dose on D11;
   - or 12-lead ECG will be checked upon early withdrawal, and 10 min rest will be required before the examination.
   - The 12-lead ECG will be collected at least 3 times during screening and baseline, with an interval of at least 1 minute between each two times. Subjects with any abnormal test will be excluded. The remain time points will be collected 3 times, with an interval of at least 1 minute between each two times, and the average value will be taken.
6. Airway assessment: Modified Mallampati scoring criteria will be used, see Appendix 4.
7. Blood routine, Urine routine, Blood biochemistry (excluding serum electrolytes), Coagulation function, Thyroid function: See Appendix 2 for detailed examination list.
8. Serum electrolytes (including potassium, magnesium, total calcium, sodium, chlorine):
   - Screening Period (D-21 to D-3);
   - Baseline Period (D-2);
   - Period I: D-1, pre-dose (within 1 h) and 2 h (±30 min) after the start of post-dose on D1;
   - Period II: pre-dose (within 1 h) and 2 h (±30 min) after the start of post-dose on D6;
   - Period III: pre-dose (within 1 h) and 2 h (±30 min), 24 h (±1 h) (D12) after the start of post-dose on D11;
   - or serum electrolyte will be checked upon early withdrawal.
9. Pregnancy test: blood pregnancy test will be performed during screening period (D-21 to D-2), Baseline Period (D-2), D12 or upon early withdrawal. Baseline (D-2) Urine pregnancy test required if blood pregnancy test is exempt .
10. COVID-19 nucleic acid detection: Throat swab samples will be taken.
11. Check-in consultation: The consultation will include medication history, medical history, surgical history, smoking history, alcohol history and allergy history.
12. ECG monitoring (including blood pressure, oxygen saturation, heart rate, respiration): to avoid potential hypoxemia due to respiratory depression, and potential hypotension and heart rate changes due to cardiac depression, subjects will receive continuous ECG monitoring from the start of each period (except moxifloxacin hydrochloride tablet dosing periods) until at least 1 h after the start of post-dose.
13. Observation of subject's consciousness: subjects will be observed for their state of consciousness from the start of each period (except moxifloxacin

hydrochloride tablet dosing periods) until at least 1 h after the start of post-dose.

14. Holter Collection, PK blood sample collection: see 1.4 for details.
15. Adverse events record, Concomitant drug, and Concomitant non-drug therapy: investigators are required to collect all AEs, concomitant drug, and concomitant non-drug therapy that occur from signing informed consent to the end of follow-up visit.
16. If a subject withdraws from the trial prior to the first dose, only vital signs are required. If a subject withdraws from the trial after receiving investigational product, the investigator should collect Holter, PK blood samples and conduct withdrawal visit as much as possible. If possible, sample collection or safety testing should occur as close as possible to the time of withdrawal.

#: Exemption from examinations during baseline period: If the following tests (physical examinations, blood routine, urine routine, blood chemistry (excluding serum electrolytes), serum electrolytes and blood pregnancy test) have been performed within 72h before baseline test， it is not necessary to repeat them at the baseline (D-2).

*: The weekly washout period is 5-7 days, SoA is now presented with a washout period of 5 days, which can be adjusted the study schedule by the site based on the final washout period.

1.4. Holter/PK Sampling/12-Lead ECG Collection Schedule

Time	Planned time point	PK[1]	Holter[2]	12-lead ECG[3]
Screening Period (D-21 ~ D-3)	/	/	/	X
Baseline Period (D-2)	/	/	/	X
Observation Period（D-1, D5 and D10）	/	/	X[4]	X
Observation Period（D1 ~ D4, D6 ~ D9 and D11 ~ D12）	Within 2 h pre-dose	/	/	X
	Within 1 h pre-dose	X	X[5]	/
	30 s after start of dosing[6]	X	X	/
	1 min after start of dosing	X	X	/
	2 min after start of dosing	X	X	/
	3 min after start of dosing	/	X	/
	5 min after start of dosing	X	X	/
	8 min after start of dosing	/	X	/
	10 min after start of dosing	X	X	/
	15 min after start of dosing	/	X	/
	30 min after start of dosing	X	X	/
	1 h after start of dosing	X	X	/
	2 h after start of dosing	X	X	X
	3 h after start of dosing	X	X	/
	4 h after start of dosing	X	X	/
	6 h after start of dosing	X	X	/

	8 h after start of dosing	X	X	/
	12 h after start of dosing	X	X	/
	24 h after start of dosing	X	X	X

1. PK blood sample collection: All subjects will be collected PK venous blood samples of approximately 3 mL within 1 h before dosing, 30 s (+6 s), 1 min (± 6 s), 2 min (± 10 s), 5 min (± 20 s), 10 min (± 1 min), 30 min (± 1 min), 1 h (± 1 min), 2 h (± 10 min), 3 h (± 10 min), 4 h (± 10 min), 6 h (± 10 min), 8 h (± 10 min), 12 h (± 10 min) and 24 h (± 10 min) after the start of dosing of each period. See 8.4 for details of collection and processing methods.
2. Holter collection：Holter collection will be collected 60 min, 45 min and 30 min pre-dose, 30 s, 1 min, 2 min, 3 min, 5 min, 8 min, 10 min, 15 min, 30 min,1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, and 24 h after the start of dosing. At 15 min prior to each Holter scheduled collection time point, subjects should rest in supine position.
3. 12-lead ECG: 12-lead ECG will be checked during screening period (D-21 to D-3), baseline period (D-2), observation period (D-1, D5 and D10), within 2 h before dosing of each period, 2 h (±30 min) and 24 h (± 1 h) after the start of post-dose of each period, or early withdrawal, and 10 min rest will be required before the examination. The 12-lead ECG will be collected at least 3 times during screening and baseline, with an interval of at least 1 minute between each two times. Subjects with any abnormal test will be excluded. The remain time points will be collected 3 times, with an interval of at least 1 minute between each two times, and the average value will be taken.
4. Subject will have Holter collection on D-1 for approximately 24 h.
5. Baseline corrected: The mean for 3 time points, 60 min, 45 min, and 30 min pre-dose on Day 1 within each respective period, will be used as baseline value.
6. The PK blood sample collection and Holter collection time point for subjects receiving HSK3486 and placebo 30 s after the start of dosing is immediately after the end of dosing.

2. INTRODUCTION

2.1. Study Rationale

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E14 recommends that a thorough QT study (TQT study) be conducted in healthy subjects for non-antiarrhythmic drugs in order to evaluate the effects of drugs on QT/QTc intervals in order to determine whether further studies on the effects on QT/QTc intervals are needed in patient populations at later stages of drug development. The QT interval is a measurement of the total duration ventricular depolarization and repolarization, (measured from the beginning of QRS complex to the end of the T wave). QT prolongation is a marker for delayed cardiac repolarization, which creates a cardiac electrophysiological environment associated with an increased risk of malignant ventricular arrhythmias, most commonly torsade de pointes (TdP). Because prolongation of the QT/QTc interval is the ECG finding associated with the increased susceptibility to these arrhythmias, an adequate investigation of the safety of pharmaceutical agent should include rigorous characterization of its effects on the QT/QTc interval.[1]

The clinical study will be performed to evaluate the effect of HSK3486 on QT/QTc interval in healthy subjects based on nonclinical/clinical studies of HSK3486, ICH E14 and food and drugs administration (FDA) submission requirements.

2.2. Background

HSK3486 is a drug developed by Xizang Haisco Pharmaceutical Co., Ltd. to be used for sedation/anesthesia induction and maintenance. The primary mechanism of action for HSK3486 is to increase chloride ion influx through the gamma-aminobutyric acid receptor subtype A ($GABA_A$)-mediated ion channels to achieve central nervous system (CNS) depression. The active ingredient of the drug, HSK3486, possesses a new structure that is analogous to propofol, These channels are also major targets of propofol action.

HSK3486 is a single-configuration chiral compound with 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. These structural improvements result in increased potency, therefore a smaller amount of drug required, less injection-site pain, and may reduce the occurrence of adverse events (AE) caused by propofol.

Based on the Investigator Brochure[2] (IB) Version 9.0 (dated 04 November 2023), the following nonclinical and clinical data are summarized, please refer to the IB for the results of specific nonclinical and clinical study results.

2.3. Nonclinical Studies

2.3.1. Effects of HSK3486 on hERG by Manual Patch-Clamp Electrophysiology

The concentration-effect relationship of HSK3486 at concentrations of 0.6, 2, 6 and 20 μM on hERG (human ether-à-go-go related gene) potassium channel (IKr, cardiac rapid activation delayed rectifier potassium current) currents stably expressed in CHO cell lines in vitro.

Results showed that 4 independent cells per test article group were tested, no precipitate was visually detected, and the IC_{50} value of HSK3486 on hERG potassium channel was higher than 16.15 μM (actual concentration of working solution after perfusion in 20 μM group) .

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 ms, 1000 ms, and 600 ms.

Results showed that both HSK3486 and propofol had no significant effect on resting membrane potential, action potential amplitude, and maximum rate of depolarization. Both drugs shortened action potential duration at 50% (APD_{50}) by about 24% and 41%, respectively, at the highest concentration. Shortening of APD was concentration-dependent but not frequency-dependent, indicating that shortened action potentials of HSK3486 are mainly manifested during Phase II within the effective concentration range, and that HSK3486 has a significantly smaller effect compared with 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 on Cardiovascular and Respiratory Systems of Dogs

The potential effect of HSK3486 on the CV and respiratory systems was assessed in Beagle dogs (given telemetry implants) given an IV injection of HSK3486 at 0 (normal saline), 1, 2, or 4 mg/kg, in a double Latin square design. QT interval, QTcF, PR interval, RR interval, QRS duration, QRS voltage, T_{p-e} interval, ST segment voltage, heart rate (HR), mean arterial pressure, systolic pressure, diastolic pressure, pulse pressure difference, respiratory rate, tidal volume and body temperature were continually monitored from 3 to 5.5 h pre-dose to 8 h post-dose. Statistical analysis and evaluation were performed on the above indicators.

Anesthetic reactions were observed within 1 h post-dose at 1, 2, and 4 mg/kg of HSK3486. The animals recovered consciousness at 9-30 min, 18-33 min, and 24-43 min post-dose, respectively.

When HSK3486 was given, statistically significant transient changes in some parameters of ECG, BP, respiration, and body temperature occurred compared with normal saline.

Heart rates at 2 and 4 mg/kg were accelerated in a dose-dependent manner, various BP indexes were lowered, and the corresponding RR interval was shortened. Changes in tidal volume of animals in each dose group was only statistically significant 2 min post-dose. No statistically significant changes were observed at other time points and there were no trended changes. Body temperatures of animals in each dose group exhibited a decreasing trend within 1 h post-dose.

No trended changes were observed in ECG parameters (QRS voltage, T_{p-e} interval, and respiratory rate). Results of separate and combined analysis of male and female animals were consistent; thus, there was no gender specificity. No statistically significant differences were observed between the various indicators at 1 to 8 h post-dose (animal regained consciousness) and the corresponding indicators at the same times with normal saline. In addition, no trended changes were observed, thus indicating that the different doses of HSK3486 had no residual effect on the animals. The above abnormal indexes were mild and transient during anesthesia.

2.3.4. Effect on Cardiovascular and Respiratory Systems of Dogs (Compared with Propofol)

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, BP, and body temperature) were continuously monitored from about 3.5 h pre-dose to about 6 h post-dose. Anesthetic effect and PK parameters were analyzed, and functional characteristics of HSK3486 and propofol were compared.

At the same time points, no trend of change was observed in the ECG parameters (QRS voltage and T_{p-e} interval) in animals treated with HSK3486 or propofol as compared with the vehicle control. Statistically significant transient changes in ECG, BP, respiration, and body temperature occurred with HSK3486 or propofol compared with the vehicle control. No trend of changes occurred in any parameter at 1 to 3 h post-dose for HSK3486 or propofol compared with the vehicle control. In conclusion, HSK3486 showed a significantly lower effect on the CV system as compared with propofol. Details are provided in Table 1.

Table 1 Maximum Changes in Cardiovascular Parameters (%) and Their Corresponding Time Points

Parameter	HSK3486		Propofol	
	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.

$\Delta\% = [(b1-b0) - (a1-a0)]/a1 \times 100$, 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.

2.4. Clinical Studies

As of August 5, 2023, 27 Phase I–III clinical studies have been completed for HSK3486. the completed 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 subjects were exposed to HSK3486, including 286 healthy subjects, 16 subjects with hepatic impairment, 20 subjects with renal impairment, 236 subjects who underwent colonoscopy, 15 subjects who underwent gastroscopy, 446 subjects who underwent induction of general anesthesia for elective surgery, 135 subjects undergoing fiberoptic

bronchoscopy, 126 subjects undergoing mechanical ventilation in ICU, and 90 subjects undergoing gynecology outpatient surgery. Results showed that HSK3486 exhibited quick onset and rapid regaining of consciousness, and had approximately 5 times the potency of propofol. The types of drug-related AE in subjects exposed to HSK3486 were consistent with those in subjects exposed to propofol. Cardiovascular-related AEs including bradycardia, and prolonged corrected QT interval (QTc) with HSK3486 were comparable to those with propofol. However, incidences of hypotension, injection-site pain and respiratory-related AEs (respiratory depression, apnea, and hypoxia), and the proportion of subjects requiring assistance in ventilation were slightly lower with HSK3486 than propofol, and the pain at the injection site was significantly lower than that in the propofol group.

2.4.1. Pharmacokinetics (PK) of HSK3486

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-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-inf} both increased by approximately 1.6 times, and the terminal half-lives of the 2 dose groups were approximately 497 min and 439 min, respectively.

In Phase I clinical study HSK3486-102, for healthy Chinese subjects infused with HSK3486 continuously for 4 and 12 h, no significant accumulation of HSK3486 was found. The primary PK parameters C_{max} and AUC of HSK3486 showed a proportional increase with the increasing dose, while the parameters such as CL, V_{ss}, and V_d showed no dose dependence. The duration of sedation effect was proven to be prolonged with the increasing administration time and exposure of HSK3486.

The CYP2B6 inducer, rifampicin, had no significant effect on the PK profiles of HSK3486.

2.4.2. Overview of Safety of HSK3486 Injectable Emulsion in Humans

Five hundred and seventy-eight (578) subjects exposed to HSK3486 had drug-related AEs out of a total of 1370 subjects (42.2%, 578/1370). Most of the AEs in subjects exposed to HSK3486 were mild to moderate, and very few were severe. These AEs resolved without treatment or after simple treatment. No subjects withdrew due to HSK3486-related AEs, and no drug-related serious adverse events (SAEs) developed in the subjects exposed to HSK3486. Drug-related AEs reported in ⩾ 1% of subjects in the pooled HSK3486 group included hypotension (20.1%), bradycardia (7.3%), injection-site pain (4.7%), dyskinesia (2.1%), dizziness (2.1%), respiratory depression (2.0%), hypoxia (2.1%), prolonged QT interval of ECG (1.50%), vomiting (1.2%), blood pressure increased (1.2%), blood pressure decreased (1.1%), apnoea (1.1%), nausea (1.0%). The incidences of injection site pain, hypotension, dizziness, and respiratory-related adverse events (respiratory depression, apnea, and hypoxia), and the proportion of subjects requiring assisted ventilation were lower with HSK3486 than propofol. The incidence of all drug-related AE is detailed in Table 2.

Table 2 Pooled Safety Summary of Drug-Related AEs (HSK3486 Group≥1%)

System Organ Class (SOC)/ Preferred Term (PT)	HSK3486 N=1370 N (%)	Propofol N=756 N (%)
Drug-related AE of at least 1 occurrence	578 (42.2)	475 (62.8)
Vascular disorders	282 (20.6)	200 (26.5)
Hypotension	276 (20.1)	194 (25.7)
General disorders and administration site conditions	77 (5.6)	218 (28.8)
Injection site pain	65 (4.7)	215 (28.4)
Cardiac disorders	119 (8.7)	69 (9.1)
Bradycardia	100 (7.3)	50 (6.6)
Investigations	109 (8.0)	73 (9.7)
Blood pressure increased	17 (1.2)	15 (2.0)
Electrocardiogram QT prolonged	20 (1.5)	11 (1.5)
Blood pressure decreased	15 (1.1)	10 (1.3)
Respiratory, thoracic and mediastinal disorders	**76 (5.5)**	**66 (8.7)**
Hypoxia	29 (2.1)	22 (2.9)
Respiratory depression	28 (2.0)	22 (2.9)
Apnoea	15 (1.1)	27 (3.6)
Nervous system disorders	**73 (5.3)**	**45 (6.0)**

System Organ Class (SOC)/ Preferred Term (PT)	HSK3486 N=1370 N (%)	Propofol N=756 N (%)
Dizziness	29 (2.1)	29 (3.8)
Dyskinesia	29 (2.1)	11 (1.5)
Gastrointestinal disorders	**36 (2.6)**	**15 (2.0)**
Vomiting	17 (1.2)	9 (1.2)
Nausea	14 (1.0)	2 (0.3)

2.4.3. HSK3486 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 subjects 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 timepoints 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 subjects 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 HR 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 HR 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 HR are as expected for anesthetic agents – a rapid increase in HR immediately following a bolus (due to the compensatory response to immediate vasodilatation and drop in blood pressure) followed by a slow decline in HR 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 HR increase). This QTc

increase was generally similar to the by-timepoint effects observed for propofol; it is difficult to determine whether this represents a true effect on cardiac repolarization due to the concomitant changes in HR 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-timepoint 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 HR, or changes in autonomic tone and hemodynamics following bolus infusions of potent anesthetics.

2.5. Benefit/Risk Assessment

2.5.1. Risk Assessment

Study drug

Safety results of completed clinical trials of HSK3486 study drug are detailed in 2.4.2.

Cardiovascular non-clinical studies of HSK3486 study drug are detailed in 2.3.

Risks of moxifloxacin hydrochloride tablets[3]

Eight (8)-methoxy fluoroquinolone antibacterial agent with broad spectrum activity and bactericidal action is moxifloxacin hydrochloride tablet, which shows broad spectrum antibacterial activity against gram positive bacteria, gram negative bacteria, anaerobic bacteria and the like in vitro. It is an antimicrobial drug that is licensed for use when other drugs fail or serious adverse reactions have been reported. Recommended dose of moxifloxacin hydrochloride tablet 0.4 g daily.

Overdose: No SAE occurred when a single oral overdose was up to 2.8 g. If acute overdose occurs, the stomach should be emptied and kept hydrated. ECG monitoring and careful observation should be performed due to the possibility of QT prolongation, and appropriate supportive treatment should be given according to the patient's clinical condition. Oral activated charcoal is effective to prevent increased systemic exposure to moxifloxacin following overdose.

According to moxifloxacin hydrochloride label, the most common adverse drug reactions (3%) in subjects receiving moxifloxacin hydrochloride were nausea, diarrhea, headache, and dizziness. Prolonged QT interval in ECG is also an adverse reaction included in moxifloxacin hydrochloride tablet. Oral administration of 0.4 g moxifloxacin hydrochloride tablets resulted in a mean QTc change of 6 ms (±26) (n=787) from pre-dose to maximum moxifloxacin hydrochloride concentration. Other adverse reactions of moxifloxacin hydrochloride tablets include: disabling and potentially irreversible serious adverse reactions, including tendinitis and tendon rupture, peripheral neuropathy and effects on the central nervous system; Tendinopathy and tendon rupture, aggravation of myasthenia gravis, allergic reactions, other serious and sometimes fatal reactions, effects on the central nervous system (adverse reactions of the central nervous system and adverse reactions of psychiatric disorders), clostridium difficile-associated diarrhea, etc.

2.5.2. Benefit and Risk management

Healthy subjects are not expected to benefit from HSK3486 in this trial, but the data from this clinical trial will be used to support further development of HSK3486 and will provide safety information for subjects and investigators participating in future clinical trials for additional indications.

Considering the safety of subjects, the commonly used clinical dose will be selected as the dose of HSK3486 and moxifloxacin hydrochloride tablet,i.e., the dose of HSK3486 is 0.4 mg/kg, and the dose of moxifloxacin hydrochloride tablets is 0.4 g, which will make the safety of this clinical trial controllable.

Possible AEs will be closely observed and monitored during the clinical trial:

- For potential hypoxemia due to respiratory depression, and potential hypotension and heart rate changes due to cardiac depression, subjects will receive continuous ECG monitoring

from the start of each period (except moxifloxacin hydrochloride tablet dosing periods) until at least 1 h after the start of post-dose;

- For cardiovascular events, inclusion/exclusion criteria should be strictly followed when screening subjects. Subjects with cardiovascular disease/condition/surgery or history of other risk factors for TdP, etc. will be excluded;
- For hepatotoxicity, inclusion/exclusion criteria should be strictly followed when screening subjects. Subjects with hepatic dysfunction will be excluded (aspartate aminotransferase (AST) and alanine aminotransferase (ALT) abnormalities, direct bilirubin above upper limit of normal (ULN), and clinically significant in the judgment of the investigator);
- For renal toxicity, inclusion/exclusion criteria should be strictly followed when screening subjects. Subjects with renal insufficiency will be excluded (estimated glomerular filtration rate (eGFR) < 90 mL/min);
- To ensure the safety of subjects, investigators should strictly follow the requirements in the trial protocol and implement the trial termination criteria and subject withdrawal criteria.

In addition, a rigorous serum pregnancy test will be performed for female subjects enrolled in this study, and subjects will be informed of the need to use highly effective contraception from signing the informed consent form (ICF) to 90 days after leaving the investigational site after the last dose in order to minimize the risk of the trial drug to the embryo or fetus.

Considering the safety of healthy subjects, it is necessary to pay close attention to the possible adverse reactions of subjects during the trial, and in order to ensure the safety of subjects, it is necessary to judge whether necessary measures need to be taken quickly after the occurrence of adverse reactions.

Risk control measures are as follows:

- Eligibility of subjects must be judged strictly according to inclusion/exclusion criteria.
- Possible risks of participating in this trial should be clearly informed to subjects in the ICF.
- Whether the first-aid drugs are complete and within the validity period shall be checked before the study and checked regularly during the test.
- Emergency plan shall be in place and improved where appropriate before starting the trial, that is, AE that may occur in this clinical trial shall be notified to the emergency department of the hospital in advance, and assistance from the emergency department shall be obtained in case of emergency.
- Adverse reactions will be monitored by the clinical center doctors and nurses throughout the trial. Physicians should not leave the Phase I clinical laboratory area until 24 hours after subject dosing and should ensure that other staff have prompt access to the supervising

physician at all other times throughout the trial. Adverse reactions of subjects need to be observed at any time during the monitoring period, symptomatic treatment should be carried out in time for adverse reactions occurring (see Appendix 5 for the recommended treatment plan for common AE), follow-up should be carried out outside the monitoring period, and in order to determine whether there are other adverse reactions.

- If the AE or its sequelae persist, the AE should be followed up even after discontinuation of the investigational product. This follow-up will continue until the adverse event is recovered, stable, reasonably explained, or the subject is lost to follow-up or refuses to provide information.
- Doctors should check the vital signs and general conditions of subjects regularly during the trial. In case of AE, measures should be taken to deal with and record them in a timely manner in order to ensure the safety of subjects.
- In the event of a SAE, emergency transport should be performed in accordance with relevant standard operating procedures (SOP).

3. STUDY OBJECTIVES AND ENDPOINT INDICATORS

3.1. 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 PK characteristics of a single IV bolus of HSK3486 for healthy subjects.

3.2. Endpoints

Primary Endpoint:

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

Secondary Endpoints:

Secondary endpoints for this clinical trial include:

- 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.
- Incidence of AE and SAE.
- Plasma concentration and PK parameters of HSK3486.

4. STUDY DESIGN

4.1. Overall Design

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

Forty eight (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 3 for dosing sequences), and the study procedure consists of 3 periods, with washout period of 5-7 days between periods.

Table 3 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)

4.2. Rationale for The Study

4.2.1. Scientific Basis for Study Design

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

- 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 assay sensitivity;
- Healthy subjects will be selected for the study population to eliminate the effect of factors 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 HR effect observed in the previous trials, the individual QT correction method (QTcI) will be used as the primary QT correct method.

4.2.2. 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.

4.2.3. Sample Size

Forty eight (48) subjects will be enrolled in this study, with the expectation that at least 42 evaluable subjects 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[4]. The sample size power calculations are detailed in Section 9.1.

4.3. 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.

5. STUDY POPULATION

Recruitment of the subjects who violate inclusion criteria and meet exclusion criteria as protocol deviations (i.e., protocol exemptions or exceptions) is not permitted.

Healthy subjects will be enrolled in this study. Subject must agree to provide written signed ICF, meet all inclusion criteria and not meet any exclusion criteria.

5.1. Inclusion Criteria

Subjects are eligible for inclusion in the study when only all of the following inclusion criteria are met:

1. Ability to understand and comply with protocol requirements and is willing voluntarily sign written ICF.
2. Healthy participants at age from 18 to 45 years old (inclusive) at Screening.
3. Male body weight ≥50 kg, female body weight ≥45 kg, with a body mass index BMI of 19~28 kg/m^2 (inclusive).
4. Left Ventricular Ejection Fraction（LVEF）≥50%.

5.2. Exclusion Criteria

Subjects will be excluded from the study if they meet any of the following criteria:

1. Past or present clinically significant systemic disease as judged by the Investigator including, but not limited to psychiatric, neurologic, pulmonary, respiratory, cardiac, gastrointestinal, genitourinary, renal, hepatic, metabolic, endocrinologic, hematological, or autoimmune disorders.
2. History of allergy to egg or egg products, soybean or soy products.
3. History of significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance. History of allergy to HSK3486 or moxifloxacin or its investigational product excipients, or history of specific allergies (asthma, urticaria, eczema, etc.), or history of tendinitis or tendon rupture due to moxifloxacin or any other quinolone drug.
4. Clinically significant infection/injury/disease within 1 month prior to dosing.
5. Current or recent (< 6 months from screening) hepatobiliary disease.
6. Current or past history of seizure disorder, including alcohol- or stimulant-related seizure, febrile seizure, or significant family history of idiopathic seizure disorder.
7. Family history of sudden death at <50 years of age.
8. History of unexplained loss of consciousness, unexplained syncope, unexplained irregular heartbeats or palpitations, clinically significant head injury.

9. Pre-existing condition interfering with normal gastrointestinal anatomy or motility, hepatic and/or renal function, or conditions that could interfere with the absorption, metabolism, and/or excretion of study drug (e.g., history of bariatric surgery or intestinal bypass surgery; simple uncomplicated appendectomies and hernia repairs are allowed, but cholecystectomy is not allowed).
10. Positive test results for hepatitis B surface antigen, hepatitis C antibody, treponema pallidum antibody, human immunodeficiency virus (HIV) antigen/antibody combination test.
11. Subjects with previous or suspected difficult airway (e.g., modified Mallampti score III-IV, congenital microglossia, mandibular dysplasia), or respiratory insufficiency, history of obstructive pulmonary disease, history of asthma, sleep apnea syndrome; history of failed tracheal intubation; history of bronchospasm requiring treatment within 3 months prior to screening; acute respiratory infection, and with obvious symptoms such as fever, wheezing, nasal congestion or cough within 1 week prior to baseline.
12. Knowledge of any kind of cardiovascular disorder/condition/procedure known to increase the possibility of QT prolongation or history of risk factors for TdP (e.g., heart failure, hypokalemia, hypomagnesemia, congenital Long QT syndrome, or family history of Long QT Syndrome.
13. Laboratory tests at screening or baseline judged clinically significant by the investigator, including, but not limited to, ALT or AST > 1.2 ×ULN (the upper limit of the reference range at screening or baseline), direct bilirubin > ULN (congenital nonhemolytic hyperbilirubinemia [e.g., suspicion of Gilbert's syndrome based on total and direct bilirubin] is not acceptable), creatine kinase (CK) > ULN (one repeat test allowed), thyroid stimulating hormone (TSH) outside normal range (0.75 to 5.6 mIU/L) , serum potassium outside normal range (3.5 to 5.3 mmol/L).
14. Rest sitting vital sign results abnormal and clinically significant at screening or baseline, ear temperature outside normal range, diastolic blood pressure ≥ 90 mmHg or systolic blood pressure ≥ 140 mmHg, HR < 55 beats/min or > 100 beats/min (test can be repeated once according to investigator's judgment).
15. Oxygen saturation (SpO_2) below 95% at baseline.
16. Abnormal 12-lead ECG at screening or baseline (any test abnormality), including any of the following:
    a) QTcF > 450 ms
    b) QRS > 110 ms
    c) PR > 200 ms
    d) Second or third-degree AV block
    e) Any rhythm other than sinus rhythm of clinical significance.

17. Estimated Glomerular Filtration Rate (eGFR) < 90 mL/min (estimated using MDRD equation).
18. Participation in another clinical study of an investigational drug (or medical device) within 3 months (or 5 half-lives, whichever is longer) prior to dosing, or previous participation in any other clinical trial related to HSK3486.
19. Donation of blood within 3 months prior to screening, plasma within 2 weeks prior to screening, platelets within 6 weeks prior to screening, or receive blood products within 2 months prior to admission to a the investigational site.
20. Sperm and egg donation program from screening period to 90 days after study end.
21. Pregnant or lactating women or those with positive pregnancy test results. Male or female subjects of childbearing potential do not agree to use an effective method of contraception from the time of signing ICF until 90 days after leaving the investigational site after the last dose (see Appendix 6 for details of specific contraceptive methods).
22. 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 or during the clinical trial.
23. Use or intend to use of any prescription, nonprescription, vitamin, herbal, or nutraceutical within 14 days prior to dosing or during the clinical trial.
24. Smoking (≥ 5 cigarettes per day) within 6 months prior to screening, or inability to quit smoking during the trial.
25. Positive alcohol breath test, or regular drinking within 6 months prior to dosing or during the trial, i.e. drinking more than 21 units (men) or 14 units (women) of alcohol per week (1 unit =360 mL beer or 45 mL spirits or 150 mL wine at 40% alcohol).
26. Positive urine drug abuse screening (morphine, tetrahydrocannabinol, methamphetamine, methylenedioxyamphetamine, ketamine), or history of drug abuse, drug dependence within 6 months prior to screening, or drug use within 3 months prior to screening.
27. Subjects who tested positive for COVID−19 during screening.
28. Eating fruits or foods affecting metabolic enzymes, such as grapefruit (citrus), pomelo, etc., within 7 days prior to screening; and not abstaining from the above beverages, fruits or foods during the study period.
29. Previous chronic excessive consumption (more than 8 cups per day, 1 cup =250 mL) of tea, coffee or caffeinated beverages, or intake of caffeine and/or purine-rich foods or beverages (e.g. coffee, tea, chocolate, caffeinated carbonated beverages, cola, etc.) within 48 hours prior to screening, or refusal to stop drinking tea, coffee and/or caffeinated beverages during the trial.

30. 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 dosing through the end-of-study visit.
31. Subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical disease (e.g., infectious disease).
32. 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 subject, poorly vascular condition, allergies to medical plastics/latex).

5.3. Lifestyle Restrictions

Subjects must be informed and reminded of the following restrictions during screening and informed consent:

5.3.1. Dietary Restrictions

- Pitaya, mango, foods or beverages containing grapefruit or pomelo are prohibited 48 h before dosing of investigational product until the end of the study.
- During hospitalization, meals should be taken at the same time as specified by the clinical trial institution, and light diet should be required.

5.3.2. Caffeine, Alcohol and Tobacco

- Caffeine or xanthine-containing foods or beverages (e.g. coffee, tea, cola drinks and chocolate) are prohibited 48 h before dosing of investigational product until the end of the study.
- Alcohol or alcoholic foods or beverages were prohibited 48 h before dosing of investigational product until the end of the study.
- Smoking is prohibited 48 h before dosing of investigational product until the end of the study.

5.3.3. Other Restrictions

- Subjects should avoid strenuous exercise (e.g., strength training, aerobic training, football, etc.) and prolonged bed rest throughout the study period.
- Subjects must not donate blood throughout the study.
- Subjects should use appropriate contraception to avoid pregnancy or to impregnate their partner from signing the ICF until 90 days after leaving the investigational site after the last dose (see Appendix 6 for specific contraceptive methods).

- When Holter collection time coincides with PK sample collection time, safety examinations time and meal time, the accuracy of Holter collection time should be ensured first, and other time should be subject to the actual operation of the investigator.
- One arm of the subject will be used for intravenous administration (except moxifloxacin hydrochloride tablet) and ECG monitoring, and the other arm will be used for PK venous blood sampling and oxygen saturation (pulse) measurement. The blood pressure measurement site at D-1 and post-dose should be ensured to be on the same arm (In exceptional cases, when the investigator determines that the same arm cannot be measured, the other arm can be measured, and the reason should be recorded at the same time).
- Subjects should avoid high-risk, high-altitude work or precision operation and driving during the whole trial period.

5.4. Screen Failure

Subjects who consent to participate (signed ICF) in the clinical trial but do not subsequently enroll in the study are considered screen failures Basic information of screening failure subjects shall be provided to ensure that the records of such subjects are clear and complete meeting the requirements of clinical trial summary report, and reply to queries from drug regulatory authorities when necessary. The information includes signed ICF, demographics, and reasons for screening failure.

Subjects who do not meet the criteria for participating in this clinical trial (screening failure) are not allowed to be rescreened. Each subject participating in screening will be assigned a screening number once and the screening number will be unique. Different screening numbers shall not be assigned to the same subject.

6. INVESTIGATIONAL PRODUCT USE AND CONCOMITANT THERAPIES

6.1. Investigational Product Use

6.1.1. Investigational Product Information

Study Drug:

HSK3486, Generic name: Ciprofol injection, provided by Xizang Haisco Pharmaceutical Co., Ltd.; Strength: 20 mL: 50 mg/vial; Storage: No more than 25 ℃ sealed storage, do not freeze.

Placebo:

HSK3486 simulator, provided by Xizang Haisco Pharmaceutical Co., Ltd.; Strength: 20 mL/vial; Storage: No more than 25 ℃ sealed storage, do not freeze.

Positive Control:

Moxifloxacin hydrochloride tablet, provided by Xizang Haisco Pharmaceutical Co., Ltd.; Strength: 0.4 g/tablet; Storage: Keep in the dark, sealed below 25 ℃.

6.1.2. Investigational Product Label

The Sponsor will design the packaging and labeling of the drug product in accordance with local laws and regulations and Good Manufacturing Practice (GMP) guidelines for clinical trials.

The label of the investigational product should include the following contents: clinical trial number, drug name, drug number, strength, dosage and administration, expiration date, storage conditions, batch number, precautions, sponsor information, etc., and will indicate “For clinical trial use only”. See the Drug Administration Manual for details.

6.1.3. Method of Administration

A: Placebo (HSK3486 simulator). IV bolus administration, the time of administration is 30 (±5) seconds.

B: moxifloxacin hydrochloride tablet 0.4g (Positive control). Oral administration with 240 mL of warm water on an empty stomach.

C: HSK3486 0.4 mg/kg (Study drug). IV bolus administration, the time of administration is 30 (±5) seconds.

The Subjects are prohibited from eating for 10 h before dosing and 4 h after dosing in each period. The subjects are prohibited from drinking water within 2 hour before and 1 hour after dosing of

each period, except for moxifloxacin hydrochloride tablet. Dosing times and food times should be approximately the same for all subjects in each period.

6.2. Management of Investigational Product

Drug packaging, transportation, distribution, storage, and recovery should meet the requirements of blinded (except moxifloxacin hydrochloride tablet) design. HSK3486, placebo and positive control moxifloxacin hydrochloride tablet required for this clinical trial will be managed according to the regulations for the administration of investigational drugs of clinical trial institutions. The clinical trial institution shall sign the drug receiving form when receiving the investigational drug, which should be signed by both the deliverer and the recipient in duplicate, and the clinical trial institution and the sponsor shall hold one copy respectively. The study institution should have a special person and a locked counter for safekeeping. The use and recovery of each drug should be recorded in a timely manner on a special record sheet, which needs to be reviewed and signed by two personnel. During and after the study, the remaining drugs, packaging and labels should be recovered in a timely manner, and should be returned to the sponsor (or its designee) according to NMPA GCP requirements after the end of the study or destroyed by the clinical trial institution after authorization by the sponsor. The clinical drugs should not be transferred to any non-clinical trial participants or third parties. The monitor is responsible for monitoring the supply, use and storage of the investigational drugs and the disposal process of residual drugs. See the Drug Administration Manual for more information.

6.3. Randomization and Blinding

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

6.3.1. Randomization

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

For subjects who have signed the 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+ three-digit Arabic number, and if there is no digit in hundred digits and ten digits, “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 numbers on Day-2 of the trial in descending order according to screening numbers. Randomization

number format is three-digit Arabic number, e.g. random number of the first subject is 001, random number of the 12th subject is 012.

Once the 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.

6.3.2. 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 trial 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 drug 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, and should not disclose the blinded information to any personnel participating in 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 blinded trial design and should be transported to the investigational site before the first visit of the first subject. In case of the following emergencies: when SAE 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 blind bottom should be provided to the Sponsor for storage.

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

6.4. Compliance

During the recruitment and screening phase, the investigator should introduce to the subjects in detail the objective of the trial, basic information of the investigational product, study protocol, trial process, dosing regimen (such as dose, mode of administration, cycle, etc.), clinical observation, frequency and process of biological sample collection, potential risks of participating in the trial, compensation and compensation, etc., so as to make the subjects fully informed and voluntarily participate in the trial and improve drug compliance; Before administration, the investigator should carefully check the subject's randomization number, drug number, dose and order of administration; After administration, the investigator should carefully count the remaining amount of investigational drug, empty drug package and administration device. After the subject takes moxifloxacin hydrochloride tablets, the subject's hands and mouth should be examined. The investigator should record the results of the subject's medication compliance.

6.5. Concomitant Treatment

During the period from screening to the completion of final clinical observations and laboratory tests, no medication other than investigational medicinal product was allowed, except in the following cases: during the trial, if the investigator considered that the subject have a disease or AE requiring necessary treatment. If treatment is required, investigators should use caution against drugs that interact with HSK3486 and moxifloxacin hydrochloride tablets (such as antacids, sucralfate, multivitamins and other products containing multivalent cations, warfarin, etc.).The investigator should identify and record any other medications taken, including drug name (generic name), dosage, date of administration, and indication for use.

7. TRIAL TERMINATION CRITERIA AND SUBJECT WITHDRAWAL

7.1. Trial termination criteria

Trial termination refers to stopping all trials halfway before the clinical trial is completed according to the protocol requirements. The purpose of trial termination is mainly to protect the rights and interests of subjects, ensure the quality of the trial and avoid unnecessary economic losses. Early termination of the clinical trial shall be notified to all parties in a timely manner. In the event of premature termination of the clinical trial, all parties to the study shall be notified promptly.

1） It is difficult to evaluate a drug if major errors in the clinical trial protocol are found in the trial;
2） Sponsor requests termination (e.g. for funding reasons, administrative reasons, unexpected safety concerns etc.);
3） NMPA or EC orders termination of the trial for some reason.

7.2. Subject Withdrawal

During the trial, a subject will not continue in the trial (i.e. withdraw from the trial) if he/she no longer performs subsequent procedures according to the protocol due to.

1） AE. The subject experiences an AE that requires early withdrawal from the trial because continued participation in the trial poses an unacceptable risk to the subject's health, or the subject is unwilling to continue participation in the trial due to an AE;

- QTc abnormalities

If clinically significant findings (including but not limited to changes from baseline in QT interval corrected using Fridericia's formula [QTcF]) are identified in ECG data after enrollment, the Investigator or qualified designee will determine whether the subject can continue in the study and whether changes in the subject's management are required. The review of the ECG at the time of collection must be documented. Any new clinically significant abnormal finding should be reported as an AE.

Investigational product should be discontinued if QTcF>500 ms or change from baseline>60 ms in the mean of 3 12-lead ECG data within 5 min of dosing, or if ventricular arrhythmia with or without symptoms occurs after dosing (with or without QTcF prolongation).

2） Major protocol deviations. After randomization, it is found that the subject does not meet the inclusion/exclusion criteria of the protocol or do not comply with the requirements of the

protocol, and continue to participation in the study will pose an unacceptable risk to the health of the subject;

3） Poor compliance of the subject, inability to receive the dose on time and in the correct amount, or use of other drugs or foods that affect ECG and PK evaluation;

4） Lost to follow-up, see Section 7.3;

5） Termination of the trial by the investigator or the sponsor for any reason (e.g., suspension or termination of the development of the study drug);

6） Other circumstances in which the subject withdrew at the discretion of the investigator. The specific reason must be recorded in the eCRF;

7） Subject voluntarily withdraws from the trial.

See SoA for data to be collected for subject withdrawal. If a subject withdraws from the trial before the first dose, only vital signs are required.

7.3. Lost to Follow-Up of Subjects

Subjects will be considered lost to follow-up when follow-up is not scheduled as per protocol and the site cannot contact them.

The following actions must be taken if a subject fails to perform protocol-required visits in the manner described in the protocol:

- Before a subject is considered lost to follow-up, the investigator or designee must make every effort to contact the subject (3 telephone calls if possible, registered mail to the subject's last known address if necessary, or other similar local means). These contact attempts should be documented in the subject's appropriate source document or eCRF.
- If the subject cannot be reached despite these efforts, he/she will be considered withdrawn from the study.
- The investigator should record the date of loss to follow-up in the eCRF.

8. STUDY ASSESSMENTS

- The study procedures and timing of this study are summarized in SoA.
- All screening assessments must be completed and reviewed to confirm that the potential subject meets all inclusion criteria and none of the exclusion criteria. The investigator will be required to record details of all subjects participating in screening and confirm their screening eligibility or record the reasons for screening failure in the eCRF. Subjects with abnormal examination items during screening period or baseline period or who do not meet the requirements for enrollment and exclusion of the study can be reexamined for confirmation.
- Adherence to study design requirements, including those specified in the SoA, is critical to study conduct.
- The test contents listed in SoA and their time requirements shall not be exempted or exempted from the provisions of the test protocol during the implementation of the protocol.
- Safety issues that may lead to withdrawal or termination of the trial should be reported back to the sponsor in a timely manner if necessary.
- In addition to the test items and frequency specified in the protocol, additional test items or frequency of tests may be performed in the study as needed.
- This clinical study is planned to last approximately 39 days, including a screening period of 19 days, baseline period and observation period of approximately 14 days (Three periods, washout period of 5 to 7 days between periods), and safety follow-up (7 (±1) day after the last dose) of 1 day.
- During the clinical trial, subjects will complete Holter collection, PK blood sampling and safety testing according to SoA.

8.1. Safety Assessments

Planned timepoints for all safety assessments are provided in the SoA.

8.1.1. Physical Examinations

Physical examination includes assessment of skin and mucous membranes, lymph nodes, head and neck, chest, abdomen, musculoskeletal, nervous system and other parts.

Planned time points for physical examinations are provided at SoA.

8.1.2. Height and Weight

Planned time points for height and weight are provided at SoA.

8.1.3. Cardiac Ultrasound

Cardiac ultrasound includes LVEF.

Planned time points for cardiac ultrasound are provided at SoA.

8.1.4. Airway Assessment

Airway assessment will be performed using the Modified Mallampati scoring criteria, see Appendix 4.

Planned time points for airway assessment are provided at SoA.

8.1.5. COVID-19 Nucleic Acid Detection

COVID-19 nucleic acid detection will be performed using throat swab sampling.

COVID-19 nucleic acid detection are provided at SoA.

8.1.6. Oxygen Saturation (pulse)

Planned time points for oxygen saturation (SpO_2) (pulse) are provided at SoA.

8.1.7. Vital Signs

Vital signs include pulse, ear temperature, respiration and blood pressure measurements. Subjects should rest in a quiet environment for at least 5 minutes prior to measurement.

Planned time points for vital signs are provided at SoA.

8.1.8. 12-lead ECG

The 12-lead ECG examination needs to be collected at least 3 times during the screening period and baseline period, with an interval of at least 1 min between each two times. Subjects with abnormal examination shall be excluded. The remain time points will be collect 3 times, with an interval of at least 1 min between every two times, and that average value will be taken.

The subject should rest in a quiet environment for at least 10 minutes prior to the examination and should be supine during the examination.

Planned time points for 12-lead ECG are provided at SoA.

8.1.9. Alcohol Breath Test

Planned time points for alcohol breath test are provided at SoA.

8.1.10. Clinical Safety Laboratory Tests

The investigator must review the laboratory report, document this review, and record any clinically significant changes occurring during the study as an AE. The laboratory reports must be filed with the source documents.

All laboratory tests with clinically significant abnormal values after the first dose should be repeated during study participation until the abnormal values return to normal or baseline values, or are no longer considered clinically significant by the investigator, or the subject is lost to follow-up.

All protocol-required clinical laboratory tests are detailed in Appendix 2 are detailed in SoA for timing and frequency and are performed in strict accordance with SoA.

All unscheduled laboratory tests determined by the investigator should be documented.

8.1.11. Pregnancy Test

Pregnancy test for female subjects.

Planned time points for pregnancy test are provided at SoA.

8.1.12. Serum Electrolytes

Serum electrolytes include potassium, magnesium, total calcium, sodium, chlorine.

Planned time points for serum electrolytes are provided at SoA.

8.1.13. ECG Monitoring

ECG monitoring includes monitoring of blood pressure, SpO_2, HR and respiration.

Planned time points for ECG monitoring are provided at SoA.

8.1.14. Observation of Subject's Consciousness

Planned time points for Observation of subject's consciousness are provided at SoA.

8.2. Adverse Events, Serious Adverse Events and Other Safety Reporting

8.2.1. Definitions

Adverse Events: All adverse medical events occurring in subjects in this clinical study, which may be manifested as symptoms, signs, diseases or laboratory abnormalities, but may not necessarily have a causal relationship with the investigational product.

AE can be aggravation of existing disease, symptom, sign, laboratory abnormality or newly diagnosed disease, newly occurring symptom, sign, laboratory abnormality, etc.

Serious Adverse Events, any of the following conditions that occur in a subject during the trial:

(1) leading to death;

(2) Life-threatening;

Note: The term "life-threatening" in the definition refers to the subject being at risk of death at the time of the event; it does not refer to the event occurring on the assumption that death would have occurred if the event had been more severe.

(3) Requires hospitalization or prolongation of current hospitalization, except in the following cases:

- Planned hospitalization or prolongation of hospitalization according to protocol requirements (e.g. for drug administration, efficacy assessment, routine procedures, physical examination, admission observation, etc.);
- Hospitalization due to medical conditions that existed prior to study participation and did not change. e.g. elective surgery/treatment scheduled prior to study participation;
- Hospitalization unrelated to adverse event, e.g. cosmetic surgery;
- Temporary observation (lasting less than 24 hours);
- Hospitalization due to changes in living circumstances or social reasons other than physical health conditions (not requiring medical or surgical intervention), e.g. lack of housing, financial constraints, caregiver rest, family environment, health insurance reimbursement, etc.).

(4) Causing permanent or severe disability/incapacity;

(5) Causing congenital malformations/birth defects;

(6) Other medically significant events.

This important medical event may be assessed as an SAE, i.e., some important medical event that do not result in death, is not life threatening, or do not result in hospitalization, but, in the

investigator's medical judgment, may harm the subject, or may require medical or surgical intervention to prevent any of the above SAEs. Examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, dyscrasia or convulsions that do not result in hospitalization, or drug dependence or addiction.

Suspected and unexpected serious adverse reaction (SUSAR) refers to a suspected and unexpected serious adverse reaction whose nature and severity of clinical manifestations exceed the available information such as the IB of the investigational drug, the package insert of the marketed drug or the summary of product characteristics.

8.2.2. Collection, Recording and Reporting of Adverse Events

8.2.2.1. Collection Period of Adverse Events

The investigator, who is the responsible subject for AE collection and recording, collects all AEs that occur from signing the ICF to the end of the follow-up visit and records the AEs on the eCRF form. Diseases and baseline abnormalities occurring from signing of ICF to administration of investigational product are generally recorded as medical history; if they meet the definition of SAE or are judged to be related to study procedures (such as study operation or adjuvant medication), they need to be recorded as AE (or SAE); after administration of investigational product until the end of all follow-up period, all adverse medical conditions are recorded as AE (or SAE).

The following events will not be recorded as AEs:

- Sleepiness, lethargy, and even loss of consciousness associated with narcotic sedation during administration (however, AEs should also be recorded if they exceed clinical expectations);
- Chronic diseases prior to participation in clinical trials;
- Elective medical examinations or surgical procedures scheduled prior to study entry;
- Overdose of investigational product or concomitant medications without any signs or symptoms.

8.2.2.2. Principles for Recording Adverse Events

All AEs should be recorded in concise, correct and standardized medical terms as far as possible, and the contents should include:(1) Description of AE and all associated symptoms;(2) start and end time of AE;(3) severity of AE;(4) treatment and intervention measures taken for AE;(5) the outcome of AE;(6) judgment on whether AE is related to investigational drug;(7) seriousness criteria of AE;(8) measures taken for investigational drug due to AE.

The Investigator should use medical terms/concepts to document AEs or SAEs, and should avoid colloquial and acronyms. Each adverse event should be recorded separately, and the completeness, accuracy and consistency of AE records should be ensured.

Specific recording principles are as follows:

(1) Determination of AE name

The name of the AE should be a medical term and a medical diagnosis should be preferred. That is, if multiple symptoms, signs, and laboratory abnormalities can be called or attributed to manifestations of a disease or impairment, this is considered an AE. If a definite diagnosis is not possible, symptoms/signs are used. When a later diagnosis becomes clear, the record is updated to replace the previous symptoms/signs with a diagnosis.

When identifying AE names, ensure that each AE name consists of a single event and that a diagnosis, sign/symptom is an AE. Therefore, when a subject experiences symptom of "vomiting and diarrhea" and records the name of his AE, it should be recorded as two AEs, such as (1) diarrhea and (2) vomiting, and two symptoms should not be recorded as one "diarrhea and vomiting". The terms hospitalization, surgery, death, etc. are not AEs in themselves, and the causes of these conditions need to be recorded as AEs. When the cause of the above condition is uncertain, the known information, such as hospitalization, death, etc., can be used as the name of the AE first, and the above information can be updated and refined in the subsequent follow-up report.

(2) Persistent or Recurrent AE

Persistent AE should be recorded only once on the eCRF. The initial severity of the event should be recorded and updated as the event worsens to record the maximum severity of the event.

Recurrent AE refer to AE that have recovered from the previous AE but occur later. The occurrence of the event should be recorded separately in the eCRF.

(3) Laboratory abnormalities or vital signs abnormalities

Abnormal laboratory values (e.g., blood biochemistry, blood routine, coagulation function, and urine routine) or other abnormal values (e.g., ECG, vital signs) that meet at least one of the following criteria are recorded as AE:

- With concomitant clinical symptoms or signs;
- Resulting in changes in the investigational product dosing regimen such as dose modification, temporary or permanent discontinuation, or discontinuation of the study;
- Other diagnostic tests or therapeutic measures (e.g. surgical intervention) are required;
- When considered clinically significant by the investigator.

It is the responsibility of the investigator to review all laboratory abnormalities and abnormal vital

signs and make medical judgment as to whether each laboratory abnormality or abnormal vital sign should be reported as an AE.

(4) Preexisting medical condition

The symptoms/signs existing in the screening period of the trial should be recorded and reported as AE when the severity, frequency and nature are aggravated after entering the trial. Changes from previous states, such as “increased headache frequency”, should be reflected in the record.

(5) Overdose

If an overdose occurs, the overdose needs to be documented and reported promptly in the medical record and appropriate eCRF. If an AE or SAE related to overdose occurs, it needs to be recorded and reported according to its corresponding requirements.

8.2.2.3. Reporting of Serious Adverse Events

In the event of an SAE, the investigator should ensure that the subject receives timely and appropriate clinical treatment.

All SAEs occurring from the time of signing ICF to the end of the follow-up visit, regardless of whether they are related to the investigational product or not, should be promptly completed by the investigator on the SAE Report Form provided by the sponsor and reported by the investigator to the sponsor and his/her designee by email within 24 hours (or no later than 72 hours in exceptional circumstances) of his/her knowledge of the SAE. If death is reported, it must also be reported to the Sponsor and its designee and the Ethics Committee within 24 hours (or no later than 72 hours in exceptional circumstances) of becoming aware of the autopsy report and the final medical report. For SAEs that occur after the end of the clinical trial or follow-up and before the review and approval conclusion is obtained, the investigator shall inform the sponsor within 24 hours (or no later than 72 hours in exceptional circumstances) after being informed.

If the Sponsor and its designee have a query about SAE reporting, the Investigator needs to respond to the query when updating follow-up information.

After receiving the SAE report form reported by the investigator, the sponsor and its designee need to make a comprehensive evaluation on seriousness, relatedness, and expectedness. After evaluation, if it is judged as SUSAR, (If the Sponsor and the Investigator cannot agree on the causality judgment between the AE and the investigational drug, and either party cannot rule out the relationship between the adverse event and the investigational drug, the report should also be expedited). The Sponsor or its designee should report the SUSAR to the drug regulatory authorities and health authorities, all investigators participating in the clinical trial, clinical trial institutions and ethics committees (if applicable) in an expedited manner according to regulatory requirements.

The original SAE Report Form and documentation of reporting to the Sponsor must be maintained at the investigational site.

8.2.3. Follow-Up of Adverse Events

For all AEs that are not completed, including AEs that the subject withdraws from the trial due to adverse events, or AEs that continue at the end of the trial, the investigator should conduct safety follow-up regularly according to the clinical actual situation until the AE is recovered, recovers to baseline level, stable status, the subject refuses to provide information, the subject is lost to follow-up, or other reasonable explanations are obtained.

Investigators should follow up SAEs and collect follow-up information to report them in a timely manner. All SAEs should be followed up until recovery, recovery to baseline level, stable status, the subject refuses to provide information, the subject is lost to follow-up, or other reasonable explanations are obtained. SAEs should be followed up as SAEs until they reach the outcomes above (if a subject reports an SAE due to hospitalization and the subject does not reach the outcome at the end of follow-up at discharge, the subject should continue to be followed up as an SAE after discharge and should not be downgraded to an AE for follow-up).

8.2.4. Severity Criteria for Adverse Events

The severity of AE is judged according to CTCAE 5.0 criteria. If AE not listed in the table occur, refer to the following criteria:

Grade 1：Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2：Moderate: minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL), Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, and managing money, etc.

Grade 3：Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL. Self-care ADL refers to bathing, dressing, and undressing, feeding oneself, using the toilet, taking medications, and not bedridden.

Grade 4：Life-threatening consequences, urgent intervention indicated.

Grade 5：Death related to AEs.

8.2.5. Criteria for Determining the Relationship between Adverse Events and Investigational Drug

According to the judgment criteria for causality between the investigational drug and AE, the correlation between AE and the investigational drug was divided into five grades: definitely related, probably related, possibly related, possibly unrelated and definitely unrelated.

Definitely related: It conforms to the known reaction type of the investigational drug, conforms to the reasonable time sequence after administration, the AE is relieved or disappeared after dose reduction or discontinuation, and the AE occurs or worsens after re-administration, which cannot be explained by the clinical state of the subject or other reasons.

Probably related: the AE is consistent with the known reaction type of the investigational drug, in a reasonable temporal sequence after administration, and reduced or disappeared after dose reduction or discontinuation, but the clinical status of the subject or other reasons may also cause the event.

Possibly related: consistent with the type of reaction known to the investigational product, consistent with a reasonable temporal sequence after administration, the AE was mitigated or not apparent after dose reduction or discontinuation, but the subject's clinical status or other causes could explain the event.

Possibly unrelated: unlikely to match the type of reaction known to the investigational product, unlikely to match the reasonable chronological sequence after administration, and the subject's clinical status or other reasons may also cause the event.

Definitely Unrelated: does not conform to the known reaction type of the investigational drug, does not conform to the reasonable time sequence after administration, the clinical state of the subject or other reasons can also explain the reaction, after excluding clinical symptoms or other reasons, the event is alleviated or disappeared.

8.2.6. Pregnancy Events

If a pregnancy (including ectopic pregnancy) occurs in a female subject or in a female partner of a male subject after administration of investigational drug until the end of the follow-up visit. The investigator must collect pregnancy events using the pregnancy event reporting form provided by the sponsor or designee and report the pregnancy event to the sponsor and designee by email within 24 hours (or no later than 72 hours in exceptional circumstances) of becoming aware.

Pregnancy itself is not considered as an AE (or SAE), but any complicated condition occurring during pregnancy or termination of pregnancy due to medical reasons will be recorded, reported

and followed up as "AE" or "SAE" according to the provisions of the protocol. Abortions for non-medical reasons are not recorded as "AEs" or "SAEs." If the outcome of pregnancy meets the criteria of SAE, such as spontaneous abortion (including threatened abortion, inevitable abortion, incomplete abortion, habitual abortion, etc.), stillbirth, neonatal death or congenital malformation, the investigator should report it according to the SAE reporting procedure.

Pregnancy events should be followed up to termination of pregnancy or 8 weeks after birth. After the pregnancy outcome is known, the investigator completes the pregnancy report form follow-up report within 24 hours of knowledge and reports to the sponsor (hospital ethics committee, if required).

All neonatal deaths occurring within the first month of life, regardless of cause, should be reported as SAE. In addition, any infant death occurring after one month of life should also be reported as an SAE if, in the opinion of the investigator, the death will be possibly related to the investigational product.

Female subjects will be withdrawn from the study immediately after pregnancy due to protocol violation; male subjects will not be required to withdraw from the study after pregnancy occurs in their partners, but informed consent of their female partners shall be obtained, and their partners shall be recorded, reported and followed up according to the handling methods of pregnancy in female subjects.

8.2.7. Drug Overdose

Overdose: Overdose is defined as intentional or accidental administration of investigational product by a subject in excess of the single dose, total dose, or frequency specified in the trial protocol, regardless of whether it is accompanied by AEs or sequelae.

If overdose occurs during the period from the first administration of investigational drug to withdrawal from the study, the investigator should fill in the Overdose Report Form and report it to the sponsor by email within 24 hours (or no later than 72 hours in exceptional circumstances) after being informed, and copy it to relevant personnel. If overdose is accompanied by or results in AE, AE information should be entered into the AE form in the original document and eCRF; if SAE criteria are met, the investigator should also report it according to SAE reporting requirements.

8.3. Holter

8.3.1. Holter Collection

In this trial, serial ECGs will be collected at Holter collection time points in SOA.

The clinical trial institution needs to ensure that the subjects use the same Holter instrument every period as much as possible, and the electrode position marking needs to be performed by the same qualified personnel every period.

The clinical trial institution needs to ensure quiet environment at each Holter collection time point.

The clinical trial institution needs to ensure ECG collection is in a separate area, isolated from other non-ECG collection electronics.

8.3.2. Analysis Methods of ECG Collected by Holter

Electrocardiogram collected by Holter 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.

8.3.2.1. 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 HR 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 HR on the continuous 12-lead ECG tracing within the 5-minute extraction window. Stability will be defined as variation in the HR 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.

8.3.2.2. Early Precision QT Analysis

Twelve（12）-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.

8.3.2.3. Cardiodynamic ECG Assessment

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-timepoint analysis to evaluate the effect of HSK3486 on the placebo-corrected change-from-baseline QTcI ($\Delta\Delta$QTcI) 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 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 9.3.2.

8.4. Pharmacokinetics

8.4.1. Blood Sample Collection and Handling

Venous blood samples will be collected in this trial according to the PK blood sampling time points in SOA, and about 3 mL venous blood will be collected at each time point. Blood samples collected after administration of HSK3486 will be used for HSK3486 drug concentration analysis. Blood samples collected after administration of placebo will be not analyzed. Blood samples collected after administration of moxifloxacin hydrochloride tablet will be analyzed only if necessary (e.g., no positive results will be observed).

For details of sample collection and processing, please refer to the Biological Sample Operation Manual.

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 4 Maximum Allowable Time Deviation for PK Blood Sample Collection

Scheduled Sampling Time	Acceptable Deviation Range
0 h（pre-dose）	Within 60min pre-dose
30 s after start of dosing	+6 s
1 min	±6 s
2 min	±10 s
5 min	±20 s

Scheduled Sampling Time	Acceptable Deviation Range
10 min≤t≤1 h	±1 min
2 h≤t≤24 h	±10 min

8.4.2. Sample Transport and Sample Analysis

For details of biological sample operation manual, please refer to blood sample preservation and transportation.

Method validation and sample analysis should follow relevant national guidelines and SOPs of test units. The contents and acceptance criteria of methodology validation shall be subject to Methodology Validation Plan. Details and acceptance criteria for biological sample determination are subject to the Biological Sample Analysis Plan.

8.4.3. Pharmacokinetic Parameters

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.

9. STATISTICAL ANALYSIS

Detailed statistical analyses are described in the Statistical Analysis Plan.

Measurement data are generally descriptive statistics with mean, standard deviation, median, minimum and maximum. For categorical variables, descriptive statistics will be performed by frequency and percentage.

All statistical analyses will be performed using SAS 9.4 or higher statistical software.

9.1. Sample Size Determination

A sample size of 48 participants is 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[5], 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 16 post-dose time points.

Sample Size Determination for Assay Sensitivity:

Based on the calculation of the sample size for a TQT study[5] with multiplicity controlled by using a Hochberg procedure[6], as the test is performed at four time points separately (1, 2, 3, and 4 hours), a one-sided 5% significance level (with adjusted one-sided significance levels of 5%, 2.5%, 1.67%, and 1.25%) is used along with a within-participant SD of 8 ms for ΔQTcI 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.

9.2. 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

Analysis Set	Definition
	for the by-time point, categorical, and morphological analyses of ECG parameters.
Safey Set (SS)	All randomized subjects who received at least one investigational product (HSK3486, moxifloxacin hydrochloride tablet, or placebo) and have post-dose safety evaluation results.
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.

9.3. Subject Disposition

The number and percentage of subjects screened, screen failure, randomization, early withdrawal and completion of the trial will be summarized, as well as the number and percentage of subjects entering each analysis set, and the reasons for dropout of dropped subjects will be summarized.

9.4. Demographic Data and Baseline Characteristics

Descriptive statistics will be performed on demographic data (date of birth, age, gender, ethnicity, work status, etc.) and baseline characteristics (height, weight, BMI, etc.). Baseline is defined as the last valid test or assessment prior to the first dose unless otherwise specified. Analyses of demographic data and baseline characteristics will be based on ITT.

9.5. Prior and Concomitant Medications

Prior medications will be defined as medications discontinued prior to the first dose of investigational product. Concomitant medications will be defined as medications that started on or after the day of the first dose or started prior to the first dose and continued after the first dose.

Drugs will be coded using the WHODrug drug dictionary. Prior and concomitant medications will be listed by subject and summarized descriptively by different investigational product.

9.6. Cardiac ECG Analysis

9.6.1. By-Time Point Analysis (Primary Analysis)

A by-timepoint 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.

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. Placebo-corrected change-from-baseline values for ECG interval parameters will also be summarized using descriptive statistics for HSK3486 at each post-dose timepoint. That is, for the placebo adjustment, the individual change-from-baseline ECG interval parameter (ΔHR, ΔQTcI, ΔQTcF, ΔPR, and ΔQRS) for placebo calculated at a specific timepoint will be subtracted from change-from-baseline ECG for the same participant on HSK3486 at the same timepoint to generate placebo-corrected change-from-baseline ECG (ΔΔHR, ΔΔQTcI, ΔΔQTcF, ΔΔPR, and ΔΔQRS). Data-based (i.e., not model-based) 2-sided 90% CI descriptive statistics will also be summarized.

The by-timepoint 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 timepoints on Day 1: categorical), treatment (HSK3486, moxifloxacin, and placebo), and time-by-treatment interaction as fixed effects, and baseline QTcI as a covariate. Subject will be treated as a random effect. 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 timepoint 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.

9.6.2. 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[6]. 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 one-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 one-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 one-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 the smallest *P* value will be tested against the one-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.

9.6.3. 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. Timepoint data will be summarized using the count of timepoints at which the assessments fall into the category and the percentage of the total number of timepoints 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 5).

Table 5 Criteria for Determining a Participant or Timepoint Categorical Outlier

ECG interval	Categorical outlier criteria
QTc (QTcI and QTcF)	Treatment-emergent value of > 450 and ≤ 480ms when not present at baseline (new onset)
	Treatment-emergent value of > 480 and ≤ 500ms when not present at baseline (new onset)
	Treatment-emergent value of > 500ms when not present at baseline (new onset)
	Increase of QTc from baseline of > 30 and ≤ 60ms
	Increase of QTc from baseline > 60ms
PR	Increase of PR from baseline > 25% resulting in PR > 200ms

ECG interval	Categorical outlier criteria
QRS	Increase of QRS from baseline > 25% resulting in QRS > 120ms
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 timepoints will be based on the number of observed timepoints across all participants within a treatment group.

9.6.4. 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.

9.7. Safety Analysis

Including the occurrence of AEs, abnormalities in physical examinations, vital signs, 12-lead ECG and clinical laboratory tests (blood routine, blood biochemistry (excluding serum electrolytes), urine routine, coagulation function, thyroid function and serum electrolytes).

9.7.1. Adverse Events

Adverse events will be coded using MedDRA, and will be summarized by SOC and the PT, respectively.

Descriptive statistical analysis of treatment-emergent adverse events (TEAE) will be performed in this trial, and AEs occurring before administration will be listed in the form of a list. Among them, TEAEs are defined as AEs that started after first dosing (inclusive) or worsened in severity after dosing. Related to the investigational product refers to the relationship between AE and the investigational product as “definitely related”, “probably related” or “possibly related”.

The number and incidence of participants with AEs will be calculated according to different investigational products: All TEAEs, TEAEs related to study drug, Grade 3 and higher TEAEs, TEAE related to study drug Grade 3 and higher TEAEs, SAEs, SAEs related to study drug, TEAE leading to withdrawal from the trial and TEAE related to study drug leading to withdrawal from the trial, TEAE leading to treatment discontinuation, TEAE related to study drug leading to treatment discontinuation, TEAE leading to death, TEAE leading to death related to study drug.

Adverse event analysis will be based on SS.

9.7.2. Laboratory Assessments

Descriptive statistics will be performed on the observed values and their changes from baseline at each evaluation time point after administration for each continuous indicator of laboratory examination, and the number and percentage of each category will be listed for qualitative indicators; and cross tabulated before and after administration. Descriptive statistics will be performed on the changes from baseline in the clinical evaluation results of laboratory tests of the most serious (most serious result after administration refers to the most serious clinical significance assessment result at each visit (including unscheduled visit) after administration of the investigational product, “abnormal not clinically significant” is more serious than “normal”, and “abnormal clinically significant” is more serious than “abnormal not clinically significant”) of different investigational products (according to the normal value range and/or investigator's judgment on clinical significance).

Analysis of laboratory indicators will be based on SS.

9.7.3. Physical Examinations

Descriptive statistical analysis will be performed on physical examination indicators at each evaluation time point. Descriptive statistics will be performed on the change from baseline in the most severe physical examination results after administration of different investigational products in the form of a cross table before and after administration (according to the range of normal values and the investigator's judgment on clinical significance).

Analysis of physical examination will be based on SS.

9.7.4. Vital Signs

Descriptive statistics will be performed on the observed values of vital signs at each evaluation time point after administration and their changes from baseline; descriptive statistics were performed on the changes from baseline in the clinical evaluation results of the most serious vital signs after administration of different investigational products in the form of cross tables before and after administration (according to the normal value range and the investigator's judgment on clinical significance).

Analysis of vital signs will be based on SS.

9.7.5. 12-Lead ECG

Descriptive statistics will be performed on the observed values of 12-lead ECG continuous indicators at each evaluation time point after administration and their changes from baseline; descriptive statistics will be performed on the changes from baseline in the most severe clinical evaluation results of 12-lead ECG after administration of different investigational drugs in the form of cross table before and after administration (according to the normal value range and/or the investigator's judgment on clinical significance).

Analysis of 12-lead ECG will be based on SS.

9.8. Pharmacokinetic Analysis

Pharmacokinetic parameter calculations and statistical analyses will be performed using WinNonlin8.3 or later and SAS 9.4 or later. Pharmacokinetic parameters of HSK3486 in humans will be calculated using a non-compartmental model.

- Based on PKCS, descriptive statistics will be performed for plasma concentrations at each time point according to scheduled blood sampling time points, including number of observed cases, arithmetic mean, standard deviation, coefficient of variation, minimum value,

maximum value, median value, etc. Mean and individual subject concentration-time profiles of HSK3486 on linear and semi-log scales.

- Based on PKPS, PK of HSK3486 in humans will be summarized, including number of observations, arithmetic mean, standard deviation, coefficient of variation, minimum, maximum, median, quartile, geometric mean, geometric coefficient of variation, etc.

Pharmacokinetic parameter analysis will be based on PKPS.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Appendix 1: Regulatory, Ethical and Study Oversight Considerations

10.1.1. Regulatory and Ethical Considerations

This clinical trial must comply with the Declaration of Helsinki, Good Clinical Practice (GCP) issued by NMPA and relevant laws and regulations. The protocol should be reviewed and approved by the Ethics Committee before implementation. The sponsor and investigator should provide the EC with the protocol and protocol amendments, informed consent form and its updates, methods and information for subject recruitment, other written materials provided to the subjects, Investigator Brochure, existing safety data, documents containing compensation information for subjects, documentation of investigator qualification, and other documents required for the EC to perform its duties. Ethics Committee approval must be obtained prior to the start of the trial. During the clinical trial, any modification of this protocol must be approved by the Ethics Committee or filed. The investigator is responsible for regularly submitting interim reports according to relevant requirements of the Ethics Committee, and informing the Ethics Committee that the trial has ended after the end of the trial.

10.1.2. Informed Consent Process

In order to protect the legitimate rights and interests of subjects, subjects must give informed consent to participate in this trial before receiving the drugs in this trial. It is the investigator's responsibility to provide the subjects or guardians and witnesses (if necessary) with a complete and comprehensive description of the objectives of the study, the manner of the study, the effects of the drug, reasonable expected benefits, possible toxic side effects and possible risks, to inform the subjects of their rights, risks and benefits, and to inform the subjects of any new information about the investigational drug in a timely manner. Subjects should be informed that this clinical trial is based on the principle of voluntary participation, that subjects can withdraw unconditionally from the trial at any time during the trial, and that subjects will not receive any adverse treatment due to withdrawal from the trial. Subjects should be informed that the investigator and sponsor have the right to read, store and statistically process the subject's trial data in accordance with relevant regulations. The informed consent form should indicate the version and version date. Only subjects who fully understand the risks and benefits of this clinical trial, as well as potential adverse events, and sign and date the informed consent form can participate in this clinical trial. If the trial protocol is modified to a certain extent during the trial, the informed consent form should be modified accordingly according to the revised contents, and the informed consent form re-signed by the subject should be obtained after approval by the Ethics Committee. Signed and dated

informed consent should be provided to the subject or his/her designated guardian and witness (if necessary).

10.1.3. Data Protection

- Participants will be assigned a unique identifier by the investigator. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent.
- The participant must be informed that his/her clinical data such as original medical records or eCRFs may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by relevant members of ethics committees, and by inspectors from drug regulatory authorities.

10.1.4. Dissemination of Clinical Study Data

All information, including but not limited to information related to the investigational product, proprietary information of the Sponsor (e.g. patent application documents, manufacturing processes, basic scientific research data, preclinical data, and product formulations), and all data generated by this study are confidential. The Investigator agrees to keep such information confidential and use it solely for the completion of the study and not for other purposes without the Sponsor's prior written consent.

The investigator should be aware that the information obtained in the clinical study will be used by the sponsor for further development of the investigational drug. Therefore, such information may be disclosed to other clinical investigators or regulatory authorities as necessary. The Investigator is obliged to provide the Sponsor with all data obtained in this study.

The results of the study will be presented in the Clinical Study Report (CSR). This report will include all data from the investigational sites, but data relevant for exploratory purposes may not be included in the CSR.

Information on this study will be published on the website of the State Drug Administration. Website: www.chinadrugtrials.org.cn.

10.1.5. Data Management

10.1.5.1. Database Creation, Data Entry and Verification

1） Establishment of database: The electronic data collection system is adopted for this project, and the sponsor or its entrusted contract research organization is responsible for the design and testing of EDC system. EDC systems are fully tested before they go live.
2） Data entry and verification: Data entry personnel trained through EDC system log in EDC system for data entry, and then clinical monitors log in EDC system for verification of original data and query results inconsistent with original records; data entry personnel check original data item by item against query list, and correct after confirmation by investigators. To ensure that the data in the database is consistent with the data in the original records.
3） The data administrator and medical personnel shall review the data one by one, and query the questionable data in EDC system by means of system query or manual query; the investigator shall answer the query in EDC system, or the investigator shall authorize the entry personnel to answer the query in EDC system after correction or confirmation in original records.

10.1.5.2. Data Lock and Export

When the database locking list is completed, the data management department submits a written application for locking the database. After approval by trial stakeholders, Data Management performs database lock-related processes. After the database is locked, the locked dataset is exported and transmitted to statisticians for statistical analysis.

10.1.6. Data Quality Assurance

All study-related subject data will be recorded on paper or eCRF. Except for data transmitted to the Sponsor or designee in electronic format (e.g., central laboratory data), the Investigator will review data entry for accuracy and correctness and complete a paper or electronic signature.

The investigator must maintain the accuracy of source documents (raw data) to support accurate entry of information on paper or in the eCRF.

The investigator must allow study-related monitoring, audits, review by ethics committees and inspection by drug regulatory authorities and provide raw data files for direct access.

Details of the monitoring strategy are provided in the monitoring plan.

The Sponsor or designee is responsible for data management for this study, including data quality checks.

Sponsor is responsible for activities delegated to other individuals (e.g. contract research organizations).

For clinical trials that are used for drug registration application, the participating institutions (including sponsors, clinical trial institutions and sample testing institutions) shall keep the necessary documents for 5 years after the approval of the investigational drug for marketing; for clinical trials that are not used for registration application, the necessary documents shall be kept for 5 years after the termination of the clinical trial.

10.1.7. Clinical Trial Quality Control and Quality Assurance

In order to ensure the quality control and quality assurance system implementation of the clinical trial, the sponsor and investigator will establish their own quality assurance system, perform their respective responsibilities, strictly follow the clinical trial protocol, adopt corresponding standard operating procedures.

10.1.7.1. Quality Assurance of Clinical Trial Process

The sponsor shall select qualified clinical trial institutions and investigators to carry out clinical trials, prepare ethical application materials according to the requirements of clinical trial institutions, and cooperate with clinical trial institutions to carry out clinical trials according to regulations and protocol requirements after obtaining ethical approval documents.

Before starting the clinical trial, investigators should receive training on the study protocol, so that investigators can fully understand the clinical study protocol and the specific connotation of each indicator. The quality control personnel of clinical trial institutions shall verify the basic conditions of the clinical trial to ensure that the clinical trial conditions can meet the requirements of the protocol. During the trial, investigators should carefully perform clinical operations and other work according to institutional SOP and trial protocol requirements, and make records truthfully, timely, completely and normatively. Quality control personnel shall conduct quality verification on test procedures and corresponding original records. At the end of the trial, the study unit collates the corresponding project documents, which are archived and stored after being checked by quality control personnel.

The quality assurance department of the investigational site conducts implement ability audits of the trials performed. When non-conformities are found, timely notify the investigator and the head of the unit for correction, and track the correction.

10.1.7.2. Quality Assurance of Sample Testing Process

The testing laboratory undertaking analysis shall establish quality assurance system, conduct quality verification in strict accordance with relevant domestic and international technical guidelines, laboratory standard operating procedures and quality control procedures, formulate verification plan, and conduct verification according to the contents of the plan. The verification contents include but are not limited to: personnel training, authorization and qualification, sample management, standard management, instrument and equipment verification, calibration and maintenance, methodology verification, sample testing, data verification, etc. The laboratory quality assurance personnel shall audit the analytical test process and test results in different categories according to the test progress and the verification results of the quality control personnel.

10.1.7.3. Quality assurance of data transmission, calculation and reporting processes

Quality control personnel of clinical trial institutions, central laboratories, data management and statistical analysis units shall check the data files related to the responsibilities of each unit, such as transmitted data files and statistical analysis data, to ensure the accuracy of data.

The sponsor shall audit the implementation of the above clinical trial process, implementation of sample testing process, recording, analysis and reporting of trial data in different fields according to the needs and in combination with the trial progress and verification results of quality control personnel/monitors.

10.1.8. Source Documents

- Source documents provide evidence of the existence of the subject and confirm the integrity of the data collected. Source documents are archived at the investigational site.
- Data transcribed from source documents to be reported in the eCRF must be consistent with the source documents, and inconsistencies must be explained. Depending on the study, the investigator may require prior medical records or transfer records. Current medical records must also be provided.
- See GCP for definitions of what constitutes source data and its sources.
- The investigator must maintain accurate documentation (source data) supporting the information entered in the eCRF.
- Source data verification will be performed by the study monitor on an ongoing basis to confirm that data entered into the eCRF by authorized site staff are accurate, complete and verifiable from source documents; that the safety and rights of subjects are protected; and that

the study is conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP and all applicable regulatory requirements.

10.1.9. Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

10.1.10. Protocol Approval and Amendments

Prior to the start of the study, the protocol and/or other relevant documents will be approved by the Ethics Committee/Drug Regulatory Authority in accordance with local legal requirements. The sponsor must ensure that all ethical and legal requirements are met prior to enrollment of the first subject.

The protocol must be strictly adhered to during the study. Protocol modifications must be made in writing prior to implementation, approved by appropriate personnel, and approved by ethics committees/drug regulatory authorities if required.

Change management of the protocol may be performed without affecting the benefit/risk ratio of the subject if no formal protocol amendment is required. Ethical review approval or filing is required prior to implementation.

All amendments will be distributed to all protocol recipients with appropriate instructions.

10.1.11. Liability and Insurance

The Sponsor will provide reasonable third-party liability insurance for subjects participating in this study in compliance with local laws and regulations.

In the event of financial loss due to personal injury and other hazards during the course of this study, the civil liability of the investigator, investigator, hospital, institution, and sponsor will be subject to applicable laws and regulations.

The sponsor will purchase insurance for this clinical study to provide insurance support to subjects participating in this study to cover financial losses incurred by subjects in the event of damage related to the investigational drug or study process.

10.2. Appendix 2: Clinical Laboratory Tests

- The tests performed at the site laboratories are detailed in Table 6.
- Specific requirements for inclusion or exclusion criteria for subjects in this protocol are detailed in Sections 5.1 and 5.2.
- Additional laboratory tests may be performed at any time during the study, as deemed necessary by the investigator or required by local regulations.

Table 6 Protocol-Specified Laboratory Tests

laboratory tests	parameters
Blood routine	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), 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), thyroid hormone (T4), free thyroxine (FT4), triiodothyronine (T3), 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)
other tests	Urine drug abuse screening: morphine, methamphetamine, ketamine, methylenedioxyamphetamine, tetrahydrocannabinol

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.

10.3. Appendix 3: Modification of Diet Inrenal Disease (MDRD) Formula

The glomerular filtration rate (GFR) calculated by the MDRD formula in mL/min/1.73 m2 is multiplied by the personal body surface area (BSA) calculated using the appropriate formula and divided by 1.73 to convert to absolute GFR in mL/min. Formula is as follows: Height and weight

$$\text{BSA} = 0.0061 \times \text{Height(cm)} + 0.0128 \times \text{weight(kg)} - 0.1529$$

Absolute GFR is uniformly used as the grading standard, expressed in mL/min, and the calculation results are rounded to 1 decimal place without rounding.

$$\text{Male: absolute GFR（mL/min）} = 175 \times (SCr)^{-1.154} \times (age)^{-0.203} \times BSA/1.73$$

$$\text{Female: absolute GFR（mL/min）} = 175 \times (SCr)^{-1.154} \times (age)^{-0.203} \times 0.742 \times BSA/1.73$$

Serum creatinine (Scr) in mg/dL, 1 mg/dL=88.4 μmol/L.

10.4. Appendix 4: Modified Mallampati Scoring Criteria

Modified Mallampati classification: The patient sits in front of the anesthesiologist, opens his mouth and stretches his tongue to the maximum (silent), and ranks the patient according to the structure of the pharynx that can be seen. The higher the Mallampati grade, the more difficult it is to intubate. Grade III, especially grade IV, belongs to difficult airway.

Grade I: soft palate, pharyngeal arch, uvula;

Grade II: soft palate, pharyngeal arch and palatal uvulas' are blocked;

Grade III, soft palate only;

Grade IV, no soft palate.

10.5. Appendix 5: Recommended Treatment Plan for Common Adverse events

Indicators	Extent	Suggested Measures
Hypotension	Mild	Close observation, no medical treatment required: if the investigator judges it as mild;
	Moderate	Routine medical treatment: 0.9% sodium chloride fluid replacement and expansion therapy;
	Severe	Proactive medical management: 0.9% sodium chloride rehydration therapy; dopamine injection 1-3ug/kg/min intravenous injection, and then adjust the speed of administration according to blood pressure;
	Life-Threatening	Rescue treatment: 0.9% sodium chloride rehydration therapy; dopamine injection 5ug/kg/min intravenous administration, and then adjust the drug speed according to blood pressure; Noradrenaline injection is dissolved in 5% glucose injection and administered initially at a rate of 16-24ug per minute. Blood pressure is monitored to adjust the rate of administration.;
Sinus Bradycardia	Mild	Close observation, no medical treatment required;
	Moderate	Routine medical treatment: atropine 0.3 mg, intravenous bolus;
	Severe	Active medical management: atropine 0.5 mg, intravenous bolus, repeat above measures if necessary;
	Life-Threatening	Rescue treatment: atropine 0.5-1 mg iv, repeated if necessary; isoproterenol injection 0.5-1 mg added to 5% glucose injection 200-300 ml iv drip. Temporary pacing therapy was given when drug therapy failed.
Pulse Oximetry Decreased	Mild	Close observation, no medical treatment required
	Moderate	Routine medical treatment: increase oxygen flow and symptomatic treatment for conditions causing hypoxia. If upper airway obstruction exists, place oral/nasopharyngeal airway; if bronchospasm occurs, immediately inject epinephrine 0.5-1 mg intravenously;
	Severe	Active medical treatment: mask positive pressure ventilation assisted breathing immediately, and symptomatic treatment for the situation causing hypoxia. If there is upper respiratory tract obstruction, place the mouth/nasopharynx airway; if there is bronchospasm, immediately push adrenal gland 0.5-1 mg; if there is laryngospasm, give skolin 0.5 mg/kg intravenous bolus (diluted to 10 mg/ml with 0.9% sodium chloride injection);
	Life-Threatening	Emergency treatment: immediate emergency endotracheal intubation (tracheotomy if necessary) and respiratory support therapy, and symptomatic treatment for conditions causing

		hypoxia. If upper airway obstruction is present, place oral/nasopharynx airway; if bronchospasm occurs, immediately intravenous bolus epinephrine 0.5-1 mg; if laryngospasm occurs, intravenous bolus of skolin 0.5 mg/kg (diluted to 10 mg/ml with 0.9% sodium chloride injection).

Note: The recommended treatment measures in this table are for reference only by investigators, and do not limit the types and doses of drugs for treatment of corresponding adverse events. Investigators can adjust the treatment measures according to the specific conditions of subjects, routine operations of clinical trial institutions and clinical habits.

10.6. Appendix 6: Contraceptive Guidance

Definitions

Women of childbearing age:

A woman is considered fertile from the start of menarche to postmenopause, unless she is permanently infertile (see below).

Additional evaluations should be considered if fertility is uncertain (e.g. amenorrhea in adolescents or athletes) and menstrual cycles cannot be confirmed prior to the first dose of investigational product.

Women will be considered to be of non-reproductive age in the following cases:

1. premenstrual
2. Premenopausal women who have one of the following conditions:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy

NOTE: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.

3. Post-menopausal female
    - A post-menopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high follicle-stimulating hormone (FSH) level in the post-menopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT).
    - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status before study enrollment.

Contraception Guidance：

a. Women who are not pregnant or breastfeeding:

- For women of childbearing age, use at least one contraceptive method (e.g., intrauterine device, barrier contraceptive method, etc.), and do not use contraceptive methods during the period from signing the informed consent form to safety follow-up;

- Premenopausal women use one of the following methods of contraception: documented tubal ligation; documented hysteroscopic tubal closure with follow-up confirmation of bilateral tubal atresia; hysterectomy; documented bilateral ovariectomy;
- Postmenopausal women, defined as spontaneous menopause lasting 12 months; estrogen replacement therapy should be discontinued prior to enrollment to confirm menopausal status;

b. Male subjects with fertile female partners:

- performs vasectomies;
- Use of male condoms and partner choice of one of the following contraceptive methods: subcutaneous contraceptive implants with an annual failure rate <1%; intrauterine devices or other intrauterine contraceptive systems with an annual failure rate <1%; oral contraceptives (progestogen alone or in combination) or progestogen injections; vaginal rings; transdermal contraceptive patches. These contraceptives must be used consistently, correctly and in accordance with product labeling. It is the investigator's responsibility to ensure that subjects understand how to use these contraceptive methods correctly.

10.7. Appendix 7: Protocol Revision History

Protocol version no.: 3.0, protocol version date: 15 March 2024.

The protocol amendment is non-substantial, which is based on the provisions of NMPA Technical Guidelines for Protocol Change during Drug Clinical Trials (Trial), which will not have significant impact on the safety of clinical trial subjects, scientificity, integrity and standardization of the trial, and reliability of trial data.

The main content of this amendment is based on protocol V2.0 (05 March 2024), which modifies the PK blood sample collection, weight measurement, and clinical manipulation in the protocol. This amendment includes the following:

Position	Pre-Revision	Post-Revision	Brief Rationale
Title Page	Version Number: V2.0, Version Date: 05 March 2024	Version Number: V3.0, Version Date: 15 March 2024	Upgrade
Signature Page	version no.: V2.0（Version Date: 05 March 2024）	version no.: V3.0 (Version Date: 15 March 2024)	Upgrade
1.3.Schedule of Activities （SoA）	Height and weight (and BMI calculation): D-1, D5, D10； 4.Height and weight (and BMI calculation): Height and weight (and BMI calculation) will be performed during screening period (D-21 to D-3), and weight will be measured after emptying urine in period I (D-1), period II (D5) and period III (D10).	Height and weight (and BMI calculation): D1, D6, D11； 4.Height and weight (and BMI calculation): Height and weight (and BMI calculation) will be performed during screening period (D-21 to D-3), and weight will be measured after emptying urine pre-dose in period I (D1), period II (D6) and period III (D11).	The revised description is to ensure accuracy of dose administered considering study drug is administered based on body weight.
1.4.Holter/PK Sampling/12-Lead ECG	Immediately after the end of dosing post-dose	30 s after start of dosing after start of dosing	1. PK blood sampling for moxifloxacin hydrochloride tablets is added considering (1) if moxifloxacin test results are

Collection Schedule	/	PK: Added 4 h after start of dosing, 8 h after start of dosing, 12 h after start of dosing, 24 h after start of dosing Holter: Added 12 h after start of dosing	unexpected, it needs to be analyzed in combination with moxifloxacin blood sample test results; and (2) different expected psychological states of subjects (blood sampling or no blood sampling) may have potential impact on ECG data. 2. PK collection and Holter collection time points is adjusted based on PK profiles of moxifloxacin hydrochloride tablet. 3. Descriptions are added to facilitate clinical manipulation.
	1.PK blood sample collection: PK venous blood samples will be collected from all subjects receiving HSK3486 and placebo within 1 h before dosing, Immediately after the end of dosing (+6 s), and 1 min (±6 s), 2 min (± 10 s), 5 min (±10 s), 10 min (±1 min), 30 min (±1 min), 1 h (±1 min), 2 h (±10 min), 3 h (±10 min) and 6 h (±10 min) after the start of post-dose of each period. See 8.4 for details of collection and processing methods. 2.Holter collection：Holter collection will be collected 60 min,45 min and 30 min pre-dose, Immediately after the end of dosing, and 1 min,2 min,3 min,5 min,8 min,10 min,15 min,30 min,1 h,2 h,3 h,4 h, 6 h, 8 h, and 24 h after the start of post-dose. At 15 min prior to each Holter scheduled collection time point, subjects should rest in supine position. ...	1.PK blood sample collection: All subjects will be collected PK venous blood samples of approximately 3 mL within 1 h before dosing, 30 s (+6 s), 1 min (±6 s), 2 min (±10 s), 5 min (±20 s), 10 min (±1 min), 30 min (±1 min), 1 h (±1 min), 2 h (±10 min), 3 h (±10 min), 4 h (±10 min), 6 h (±10 min), 8 h (±10 min), 12 h (±10 min) and 24 h (±10 min) after the start of dosing of each period. See 8.4 for details of collection and processing methods. 2.Holter collection：Holter collection will be collected 60 min, 45 min and 30 min pre-dose, 30 s, 1 min, 2 min,3 min,5 min,8 min,10 min, 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, and 24 h after the start of dosing. At 15 min prior to each Holter scheduled collection time point, subjects should rest in supine position. ... 6.The PK blood sample collection and Holter collection time point for subjects receiving HSK3486 and placebo 30 s after the start of dosing is immediately after the end of dosing.	
5.3.3.Other Restrictions	/	• One arm of the subject will be used for intravenous administration (except moxifloxacin hydrochloride tablet) and	The description is added mainly considering clinical operation.

		ECG monitoring, and the other arm will be used for PK venous blood sampling and oxygen saturation (pulse) measurement. The blood pressure measurement site at D-1 and post-dose should be ensured to be on the same arm (In exceptional cases, when the investigator determines that the same arm cannot be measured, the other arm can be measured, and the reason should be recorded at the same time).	
6.3.2.Blinding	To maximize blinding, a blinded trial arm will be established. Blinded investigators do not participate in the implementation process of clinical trials and are only responsible for judging the data generated by the trials, such as the relevance of AEs.	To maximize blinding, a blinded trial 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 drug in the trial.	Amendments to the language description.
8.4.1. Blood Sample Collection and Handling	Venous blood samples will be collected in this trial according to the PK blood sampling time points in SOA. Blood samples collected after administration of HSK3486 will be used for HSK3486 drug concentration analysis.	Venous blood samples will be collected in this trial according to the PK blood sampling time points in SOA, and about 3 mL venous blood will be collected at each time point. Blood samples collected after administration of HSK3486 will be used for HSK3486 drug concentration analysis. Blood samples collected after administration of placebo will be not analyzed. Blood samples collected after administration of moxifloxacin hydrochloride tablet will be analyzed only if necessary (e.g.,	The revisions are made primarily to take into account the purpose of the blood sample testing and the subsequent process of the test.

		no positive results will be observed).	
	Table 4 Maximum Allowable Time Deviation for PK Blood Sample Collection Immediately after the end of dosing 2 min≤t≤5 min 2 h≤t≤6 h	Table 4 Maximum Allowable Time Deviation for PK Blood Sample Collection 30 s after start of dosing 2 min； 5min（±20 s）； 2 h≤t≤24 h	The revisions are made primarily based on the PK characteristics of moxifloxacin hydrochloride tablet as well as clinical operations.

Protocol version no.: 2.0, protocol version date: 05 March 2024.

The protocol amendment is non-substantial, which is based on the provisions of NMPA Technical Guidelines for Protocol Change during Drug Clinical Trials (Trial), which will not have significant impact on the safety of clinical trial subjects, scientificity, integrity and standardization of the trial, and reliability of trial data.

The main content of this amendment is based on protocol V1.0 (03 January 2024), which modifies "double-blind" in the trial protocol to "blinded". This amendment includes the following:

Position	Pre-Revision	Post-Revision	Brief Rationale
Title Page	Version Number: V1.0, Version Date: 03 January 2024	Version Number: V2.0, Version Date: 05 March 2024	Upgrade
Signature Page	version no.: V1.0（Version Date: 03 January 2024）	version no.: V2.0 (Version Date: 05 March 2024)	Upgrade
Entire Text	double-blinded; double-blind	blinded	The revised description is to consider that the trial drug has a

			sedative effect and cannot be completely double-blind.

11. REFERENCES

1. Guidance for Industry. E14: Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs. FDA, October 2005.
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5. Zhang J, Machado SG. Statistical issues including design and sample size calculation in thorough QT/QTc studies. J Biopharm Stat. 2008;18(3):451-467.
6. Hochberg Y. A sharper Bonferroni procedure for multiple tests of significance. Biometrika. 1988; 75, 800–802.