A randomized, double-blind, placebo-controlled, single-dose and multiple-dose escalation Phase I clinical first-in-human trial evaluating the safety, tolerability, and pharmacokinetics of IPG11406 in healthy adult subjects

Clinical Trial Protocol

Plan ID:	**IPG11406-C001**
Solution version:	**V1.0**
Version date:	**07 April 2023**
Sponsoring organization:	Nanjing Immunophage Biotech Co., Ltd.

Sponsor's protocol signature page for the first-phase human trial of IPG11406, a randomized, double-blind, placebo-controlled, single-dose and multiple-dose escalation study evaluating safety, tolerability, and pharmacokinetics in healthy adult subjects

I will conscientiously fulfill the sponsor's responsibilities in accordance with Good Clinical Practice (GCP) and all applicable regulatory requirements, including the initiation, application, organization, and funding of this clinical trial. I shall assume legal liability for the medical expenses and provide appropriate economic compensation for any trial-related harm or death of subjects during the clinical trial, and provide legal guarantees to the investigators. I hereby agree to conduct this clinical trial in accordance with the design and provisions of this protocol (Version No.: V1.0, Version Date: April 7, 2023).

personal name：_____

Signature: _____

date：_____

Sponsoring Organization: Nanjing Immunophage Biotech Co., Ltd.

A first-phase, randomized, double-blind, placebo-controlled, single-dose and multiple-dose escalation clinical trial evaluating the safety, tolerability, and pharmacokinetics ofIPG11406 in healthy adult subjects

Principal Investigator's Protocol Signature Page

I have read and understood this trial protocol (Version: V1.0, Date: April 7, 2023) and agree to conduct this trial in accordance with the protocol and related annexes. I will provide a copy of the protocol to my research team and engage in discussions with them to ensure their full understanding of the trial. I am aware that the trial may be terminated or enrollment may be halted at any time due to certain predefined reasons, and the trial may also be discontinued to protect the rights and interests of the participants. I consent to conduct this trial in strict compliance with Good Clinical Practice (GCP).

Meanwhile, as the principal investigator of this trial, I will coordinate all participating researchers in accordance with Good Clinical Practice (GCP) requirements.

personal name：_____

signature：_____

date：_____

Test Center Name: _____

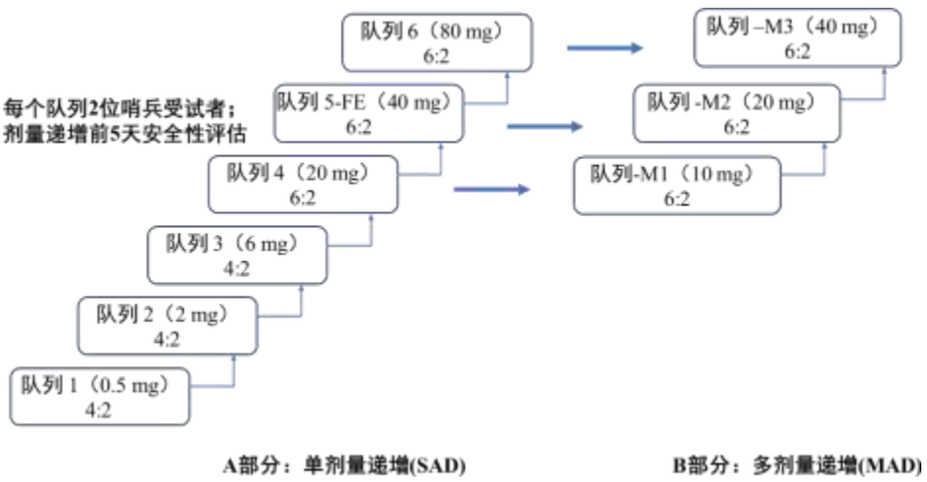
Catalogue

Abbreviation

Phrases and specialized terms	Explain
AE	Adverse event
ALB	Albumin
ALT	Alanine aminotransferase/Aspartate aminotransferase
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase/Aspartate aminotransferase
$AUC_{0\sim t}$	Area under the concentration-time curve from zero to the final measurable concentration
$AUC_{0\sim\infty}$	Area under the concentration-time curve from zero to infinity
BMI	Baric index
BP	Blood pressure
BUN	Usea nitrogen
C_{max}	The highest observed plasma concentration
$C_{ss,\ max}$	Steady-state peak concentration
$C_{ss,min}$	Steady-state valley concentration
$C_{ss,av}$	Mean steady-state plasma concentration
CK	Serum creatine kinase
CL/F	Apparent clearance rate/F
CRE/Cr	Creatinine
ECG	Electrocardiogram
ECHO	Echocardiogram
GCP	Good Clinical Practice (GCP)
γ-GT/GGT	γ-glutamyl transferase
HBsAg	Hepatitis B surface antigen
HB	Hemoglobin
HCT	Hematocrit
HCV	HCV
HIV	Human immunodeficiency virus
MRSD	Maximum recommended starting dose
NOAEL	Non-toxic dose
PLT	Blood cells
RBC	RBC
SD	Standard deviation
SAE	Serious adverse events
SOP	Standard practice
TBA	TBA
T-bili/TBIL	Total bilirubin
Urea	Urea
ULN	Upper limit of normal value

Summary of Trial Protocol

Study title	A randomized, double-blind, placebo-controlled, single-dose and multiple-dose escalation Phase I clinical first-in-human trial evaluating the safety, tolerability, and pharmacokinetics of IPG11406 in healthy adult subjects
Protocol number	IPG11406-C001
Version/ Date	1.0 Version, April 7, 2023
Sponsor	Nanjing Immunophage Biotech Co., Ltd.
Study drug	IPG11406 tablets, 0.5 mg/tablet, 10 mg/tablet, 40 mg/tablet
Studyphase	I
Indication	Inflammatory bowel disease (IBD), multiple sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA)
Purpose of research	**A. Partial Single-Dose Increment (SAD)** ✓ fundamental purpose: 1. Evaluation of the safety and tolerability of a single oral dose ofIPG11406 ✓ secondary objectives: 1. Evaluation of the pharmacokinetic (PK) characteristics of single-dose oral IPG11406 2. Evaluation of Food Effects on the Pharmacokinetics of Single-Dose Oral IPG11406 **A. Partial Single-Dose Increment (SAD)** ✓ exploratory purpose Evaluation of the pharmacokinetic (PK) characteristics of single-dose oral IPG11406 **Part B – Multiple Dose Increment (MAD)** ✓ fundamental purpose: 1. Evaluation of the safety and tolerability of high-dose oral IPG11406 ✓ secondary objectives: 1. Evaluation of the pharmacokinetic (PK) characteristics of the oral IPG 11406 in multiple-dose administration
Study end point	**Part A (single dose escalation)** ✓ Primary study endpoint: 1. Safety indicators: adverse events, vital signs, and clinical laboratory parameters; ✓ secondary end points: 1. Pharmacokinetic parameters:C_{max}， T_{max}， $t_{1/2}$,AUC_{0-t},AUC_{0-inf}, CL/F; ✓ exploratory end point 1. Measurement of IPG11406 parameters in urine: Renal clearance (CLR_{0-t}), cumulative fraction excreted in urine from time 0 to t (Ae_{0-t}), fraction of excreted dose in urine from time 0 to t (Fe_{0-t}) **Part B (Multiple Dose Increases)** ✓ Primary study endpoint: 1. Safety indicators: adverse events, vital signs, and clinical laboratory parameters; ✓ secondary end points: 1. Pharmacokinetic parameters:$C_{ss,max}$,$t_{1/2}$,$T_{ss,max}$,$AUC_{0-\tau}$,CL_{ss}/F, accumulation index $Rac_{(AUC)}$, $Rac_{(Cmax)}$.
Trial population	Health volunteer
Sample capacity	Approximately 66 healthy subjects: 42 in part A and 24 in part B.

Trial period	PART A (excluding the food effect cohort): up to 36 days (screening period: 28 days; single-dose administration period: 1 day; safety evaluation + follow-up: 7 days) Part A (food effect cohort): ≥41 days (screening period: ≤28 days; single-dose administration period (fasting): 1 day; washout period ≥4 days; single-dose administration period (postprandial): 1 day; safety evaluation + follow-up: 7 days) Part B: Maximum 45 days (screening period: 28 days; multiple-dose administration period: 10 days; safety evaluation + follow-up) Visit period: 7 days
Research design	Randomized, double-blind, placebo-controlled, single-dose and multiple-dose escalation
Experiment design	**Overview** This study is the first Phase I clinical trial conducted in humans, randomized, double-blind, placebo-controlled, with single-dose and multiple-dose escalation, aimed at evaluating the safety, tolerability, and pharmacokinetic characteristics of IPG11406 in healthy adult subjects administered with single-dose escalation and multiple-dose escalation. This study was divided into two consecutive phases: Phase A, a single-dose escalation (SAD), and Phase B, a multiple-dose escalation (MAD). Both phases included a 28-day screening period and a 7-day safety evaluation and follow-up after the completion of the dosing period. The design pattern of single-dose escalation in part A and multi-dose escalation in part B is shown in the figure below.  **Overview of part A of the study:** In this study, six dose groups will be administered: 0.5 mg (Cohort 1),2 mg (Cohort 2),6 mg (Cohort 3),20 mg (Cohort 4),40 mg (Cohort 5), and 80 mg (Cohort 6). In Cohorts 1 to 3, each cohort will consist of 6 participants, with 2 receiving placebo and 4 receiving IPG11406. In Cohorts 4 to 6, each cohort will consist of 8 participants, with 2 receiving placebo and 6 receiving IPG11406. All participants will undergo screening 28 days prior to administration. Eligible participants will enter the research center 1 day before the 5-day administration and observation period (with a 6-day hospital stay). This part of the study plans to enroll approximately 42 healthy adult participants.

	In each dose group, 2 sentinel subjects will be randomly selected and administered the drug 48 hours prior to the scheduled dose. One of these subjects will receive IPG11406, while the other will receive a placebo. If neither of the two sentinel subjects exhibits any significant safety issues within 48 hours, the remaining subjects will continue to receive the drug. After completing all safety assessments and PK sampling analyses, the subjects will be discharged on day 5 and undergo follow-up on day 8. One cohort (provisional cohort 5, subject to adjustment based on decisions from the SMC meeting) will be selected to evaluate the effects of food on pharmacokinetic parameters. Subjects in this cohort will receive a second dose of IPG11406 or placebo postprandially on day 5 after completing the first dose, following a standard high-fat meal (total caloric intake approximately 800-1000 kcal, with approximately 50% of total calories derived from fat). After each cohort completes 5 days of administration and evaluation (with the food-influence cohort evaluated on day 10), the Safety Monitoring Committee (SMC) will assess the safety and tolerability of IPG11406 based on all accumulated safety data (including follow-up data) and available pharmacokinetic (PK) data from the blinded study. Based on the evaluation results of safety and tolerability, the SMC will determine whether to proceed with administration of the next dose group. **B Summary of the study** After the safety and tolerability evaluation of IPG11406 in Part A (SAD) is completed, the Part B study with multiple dose escalation will commence. The Safety Monitoring Committee will determine the appropriate dose levels for Part B based on the safety and tolerability data from Part A. In Part B, three dose escalation levels will be administered to evaluate the safety and tolerability of IPG 11406. The study plans to enroll approximately 24 healthy adult subjects, divided into three dose groups, with 8 subjects in each cohort. Subjects will sequentially receive doses of 10 mg (cohort M1),20 mg (cohort M2), and 40 mg (cohort M3). Among the 8 subjects in each cohort, two will receive the placebo control, while the remaining 6 subjects will receive IPG11406. All subjects will undergo screening 28 days prior to administration. Eligible subjects will enter the research center one day in advance and subsequently undergo a 14-day dosing and observation period. The first dose will be administered on the morning of day 1, with daily dosing continuing for 10 consecutive days. During the dosing period, blood samples will be collected as planned to evaluate the pharmacokinetic (PK) parameters ofIPG11406. After completing all safety assessments and PK sampling analyses, the subjects were discharged on day 14, with follow-up conducted on day 3 post-discharge. After completing all safety assessments and PK sample analyses, subjects will be discharged on day 14 and followed up on day 3 post-discharge. Following the completion of 14 days of dosing and evaluation in each cohort, the Safety Monitoring Committee (SMC) will evaluate all accumulated safety data (including follow-up data) based on the blinded protocol and available

PK data were collected to evaluate the safety and tolerability of IPG11406, and the decision on whether to proceed with the next dose cohort was based on the evaluation results.

Rationality of initial dose selection

According to the "Guidelines for Estimating the Maximum Recommended Starting Dose of a Drug in the First Clinical Trial of Healthy Adult Volunteers," IPG11406 is a novel mechanism compound. To calculate the Minimum Recommended Safe Dose (MRSD) of IPG11406, we selected 1/10 of the dose at which no toxic effects were observed (NOAEL) in nonclinical safety evaluations in beagle dogs or SD rats after 28 days of repeated administration, and considered the pharmacologically active dose (PAD) in mouse models. Based on the pharmacologically active dose, we chose 2 as the safety factor, resulting in a calculated MRSD of 0.5 mg for IPG 11406. Considering that rats are a sensitive species, the starting human dose was calculated as 1/10 of the NOAEL in rats after 28 days of repeated administration, yielding 24 mg. Therefore, 0.5 mg was selected as the starting ramp-up dose for humans, and this dose was deemed to have good safety and tolerability in humans.

Calculation of the Maximum Recommended Starting Dose (MRSD)

Computational method	Genera	Dosage / mg/kg	Equivalent human dose/mg/kg	Safety facto	MRSD*/mg
PAD	Mouse	0.1，BID	0.018	2	0.5
NOAEL	Rat	20	4.04	10	24
	Dog	15	8.73	10	52

* Assume the body weight is 60 kg; BID: twice daily.

Selection criteria

1. Healthy volunteers aged between 18 and 50 years old, both male and female, are required. Participants must undergo medical history investigation, physical examination, vital signs examination, electrocardiogram, chest X-ray examination, and laboratory tests during the screening period to ensure good health.
2. The body mass index (BMI) is between 18 and 32, calculated as: BMI= weight (kg)/ height ^{2}(m ^{2});
3. Participants were required to be in a non-menstrual, non-pregnant, and non-lactating period during the trial and to agree to have no childcare plans for the next 6 months.
4. Physical health, defined as: detailed and clear medical history, comprehensive physical examination (including blood pressure and pulse rate, laboratory tests, and 12-lead electrocardiogram) with no clinical abnormalities detected;
5. After 10 minutes of supine position testing, vital signs should be within the following range:

   95 mmHg <systolic blood pressure (SBP) <140 mmHg

   45 mmHg <Diastolic Blood Pressure (DBP) <90 mmHg

   45 bpm <Heart Rate (HR) <90 bpm
6. After 10 minutes of resting supine position, 12-lead ECG readings: PR interval <120 ms <220 ms, QRS complex <120 ms, QTc (Fridericia recommended algorithm) ≤450 ms, normal ECG; or abnormal ECG results deemed clinically insignificant by the investigator.
7. Laboratory test results must fall within the normal range (or within the established screening threshold) or show no clinically significant abnormalities; however, serum creatinine, alkaline phosphatase, and liver enzymes (aspartate aminotransferase,

	Alanine aminotransferase (ALT) should not exceed the upper limit of the laboratory normal range. Total bilirubin should not exceed 1.5 times the upper limit of the normal range for conjugated bilirubin (unless the subject has a history of Gilbert syndrome). 8. Willing and able to comply with all medical visitation, medication administration, and laboratory testing protocols, agree to take the oral test medication, and meet other study requirements 9. Contraceptive requirements for female subjects: a. Fertile female participants: Must undergo a urine pregnancy test during the screening period with a negative result, and agree to use effective contraception for 3 months after signing the informed consent until the last dose of the investigational drug. Contraception must always be strictly followed according to the instructions of the contraceptive product and the investigator. Effective contraception includes: complete abstinence, intrauterine device (IUD) or intrauterine contraceptive system (IUS), double-barrier method (e.g., spermicide plus male condom, female condom, diaphragm, cervical cap, or intrauterine device), or a partner who has undergone vasectomy and whose semen cannot be detected for sperm. b. Fertile women: This includes postmenopausal women (with complete cessation of menstruation for ≥1 year) or women with surgical records of hysterectomy, bilateral oophorectomy, or bilateral salpingectomy (as opposed to tubal ligation). Fertile women do not need to adhere to the listed contraceptive measures. 10. Contraceptive requirements for male subjects: Male subjects must agree to use the listed effective contraceptive methods or have undergone vasectomy within 3 months after signing the informed consent and receiving the last dose of the investigational drug. 11. Participants must provide informed consent for this study prior to enrollment and voluntarily sign a written informed consent form.
Exclusion criteria	1. History of severe cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immune, dermatological, neurological, or psychiatric disorders. 2. Abnormal chest X-ray findings. 3. Recurrent headache and/or migraine, recurrent nausea and/or vomiting (vomiting only: more than twice a month). 4. Blood donation within 2 months prior to inclusion is permitted, with no volume restrictions. 5. Symptomatic or asymptomatic orthostatic hypotension: defined as a drop in systolic blood pressure of ≥30 mmHg within 3 minutes when changing from supine to standing position. 6. History of drug allergy or confirmed allergic disease. 7. Known to be allergic to any component of the pharmaceutical preparation. 8. History of drug or alcohol abuse (regular daily alcohol intake exceeding 40 mL). 9. Heavy smokers (smoking 5 or more cigarettes per day). For light smokers (smoking fewer than 5 cigarettes per day), smoking should be discontinued during the trial period. 10. Individuals who consume excessive amounts of tea or coffee (more than 8 cups per day). 11. Take any medication within 14 days before inclusion or within 5 times the half-life or pharmacodynamic half-life of the medication; 12. Eat any citrus fruit (e.g. grapefruit, orange) or juice in the 5 days before selection.

	13. Any vaccination within 28 days prior to inclusion and any injection of a biological agent (antibody or its derivative) within 4 months. 14. According to the investigators' assessment, the subjects may have poor compliance during the study or may not be able to cooperate due to language issues or intellectual developmental delays. 15. Participants were registered or had previously participated in any other clinical study involving a drug clinical trial or any other type of medical research within 3 months prior to enrollment or within the 5 half-life period (whichever is shorter). 16. Participants who could not be contacted in an emergency. 17. Researchers or associate researchers, research assistants, pharmacists, study coordinators, or other staff members who are directly or indirectly involved in the study, or anyone associated with the study site (including employees or immediate family members), investigators, or the sponsor. 18. Any of the following tests are positive: hepatitis B surface antigen (HBsAg), hepatitis B core antigen (anti-HBcAg), anti-HCV antibody, anti-human immunodeficiency virus 1 and 2 antibody (anti-HIV1 and anti-HIV2 Ab), or Treponema pallidum antibody. 19. Positive urine drug screening (amphetamines/methamphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine, opioids). 20. Alcohol test positive. 21. Subjects with difficulty in venous blood collection.
Medication	Administer orally on an empty stomach (defined as fasting for 10 hours overnight). Swallow the test drug with a specified volume of warm water, and the investigator must inspect each subject's mouth to ensure the drug is swallowed.
Dose escalation and discontinuation rules	**Dose escalation rule** In both Part A and Part B, the decision to increase the drug dose will be based on a review of all available safety information, including adverse events, electrocardiograms (ECGs), vital signs, clinical laboratory test results, and available pharmacokinetic data. The dose may be adjusted based on safety and pharmacokinetic data observed during the study. The dose escalation from Cohort 1 to Cohort 2 will be entirely based on safety and tolerability data. The food effect cohort will be based on all safety and tolerability data as well as the pharmacokinetic data of the first dose at that level. The decision to escalate the dose will be made by the Safety Monitoring Committee (SMC), and all dose escalation meetings and decisions will be formally documented. **Discontinuation Rules** 1. Adverse events of grade 3 or higher that cannot be ruled out as related to IPG11406 were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE). 2. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≥3×upper limit of normal (ULN), total bilirubin ≥2×ULN 3. QTcF⩾500 ms

	4. If a subject meets any of the above criteria, the subject will discontinue administration; additionally: For each cohort: If two or more subjects at each dose level meet any of the above criteria, the sponsor will unblind the relevant subjects. • If two or more subjects received IPG11406 rather than placebo, after review of all data, the sponsor and investigators may choose the approach they consider safe: 1) all subjects in the dose group will discontinue administration; 2) Part A or Part B of the study will be discontinued. • if a placebo subject is among two or more subjects, the sponsor and investigator may choose the approach they consider safe after review of all data: 1: continue with the original protocol; 2: reduce the dose or frequency of administration; or 3: stop part A or part B of the study.
Pharmacokinetic blood sampling	The pharmacokinetic evaluation method for IPG11406 involves parameter comparison between dose groups. Due to the lack of clear clinical prediction for this novel drug, adjustments to blood collection time points may be required based on actual data obtained. The following time points are provisionally designated for blood sample collection: **A Partial blood collection time points (excluding the food-influence cohort):** **Day 1:**before administration (within 1 h); 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 9, 12,24,36,48,72, and 96h after administration. **A Partial blood collection time points (food-influenced cohort):** **Days 1 and 6:**before administration (within 1 h); 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 9, 12,24,36,48,72, and 96h after administration. **B Partial blood collection time points:** **Day 1:**before administration (within 1 h), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 9, 12, and 24h after administration. **Day 4, Day 6, Day 8:**before administration (within 1h before administration). **Day 10:**before administration (within 1h before administration), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 9, 12,24,36,48,72, and 96h after administration.
Pharmacokinetic evaluation	The following pharmacokinetic parameters of IPG11406 in human were calculated by WinNonlin non-ventricular model. 1. Terminal half-life ($t_{1/2}$); 2. area under the curve (AUC) of the drug; 3. apparent volume of distribution (Vss); 4. Systemic clearance (CL/F): 5. Peak blood concentration C_{max} and time to peak T_{max}

	6. the valley concentration, steady-state distribution volume, and accumulation index (AI); 7. Other analyzable PK parameters, etc.
Tolerability and safety evaluation	Adverse events occurring during the entire study period were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0. The assessment included vital signs, physical examination, 12-lead electrocardiogram (ECG), 24-hour electrocardiographic monitoring, clinical laboratory tests (complete blood count, blood biochemistry, urinalysis, coagulation function tests, etc.), adverse events, and serious adverse events.
Statistical method	Upon finalization of the trial protocol, statistical specialists shall collaborate with principal investigators to develop the statistical analysis plan. Statistical analysis will be performed using SAS9.4 (or later versions) statistical software, while non-ventricular pharmacokinetic models in WinNonlin pharmacokinetic software will be employed to calculate key pharmacokinetic parameters for each subject. Measurement data will be described statistically using case numbers, mean, standard deviation, median, maximum value, and minimum value. Categorical or ordinal data will be described using frequency and frequency distributions. **Safety evaluation** Safety evaluation will be performed on the safety dataset. For physical examination, 12-lead electrocardiogram (ECG), 24-hour ECG monitoring, and clinical laboratory tests (including complete blood count, biochemical blood tests, urinalysis with sediment, coagulation function, etc.) were conducted. All completed tests and descriptive statistics were listed in a cross-tabulation format before and after medication administration (based on clinical judgment). Abnormal findings from ECG, physical examination, and laboratory tests at each time point were documented in a checklist. Vital signs (blood pressure, body temperature, pulse, respiration) were described, including values at each time point and their relative changes from baseline. For example, the relationship between ECG intervals and baseline changes in drug concentrations was analyzed to evaluate the exposure-response relationship. Summarize all adverse events and the incidence of study drug-related adverse events according to the CTCAE 5.0 standard levels, and summarize the incidence of adverse events with toxicity grade greater than level 3 and study drug-related adverse events. The types of adverse events and their relationship to the study drug will be described in a list. List all adverse events, study drug-related adverse events, events leading to discontinuation, and serious adverse events. **Pharmacokinetic evaluation** The pharmacokinetic parameters of the non-ventricular model were estimated and analyzed using WinNonlin (version 6.4 or later). Key pharmacokinetic parameters were calculated to comprehensively reflect the drug's absorption, distribution, metabolism, and excretion characteristics in humans. These parameters include but are not limited to:$t_{1/2}$, AUC, Vss, CL/F,C_{max}, and T_{max}. Descriptive statistical analyses were performed on pharmacokinetic parameters across different dose groups, including arithmetic mean, standard deviation, coefficient of variation, median, maximum value, minimum value, and geometric mean. In multi-dose escalation studies, the primary pharmacokinetic parameters for different dose groups were evaluated.

	The pharmacokinetic parameters were analyzed for linear pharmacokinetics, and the drug accumulation was studied based on the peak and trough concentrations over multiple cycles.
Duration of study	Annum

Table 1 A Flowchart of the Study (excluding the Food Effect Cohort)

1.

-
-
-