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Pharmacokinetics, Metabolism, Excretion and Safety of Iruplinalkib (WX-0593), a Novel ALK Inhibitor, in Healthy Subjects: a Phase I Human Radiolabeled Mass Balance Study

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Abstract

Background: Iruplinalkib is a novel anaplastic lymphoma kinase (ALK) inhibitor for the treatment of ALK-positive crizotinib-resistant NSCLC.

Research design and methods: A single oral dose of 120 mg/3.7 MBq [^{14}C]iruplinalkib was administered to healthy subjects. Blood, urine and fecal samples were collected and analyzed for iruplinalkib and its metabolites. The safety of iruplinalkib was also assessed.

Results: Iruplinalkib was absorbed quickly and eliminated slowly from plasma, with a T_{max} of 1.5 h and $t_{1/2}$ of 28.6 h. About 88.85% of iruplinalkib was excreted at 312 h, including 20.23% in urine and 68.63% in feces. Seventeen metabolites of iruplinalkib were identified, and M3b (demethylation), M7 (cysteine conjugation), M11 (oxidative dehydrogenation and cysteine conjugation of M3b) and M12 (oxidative dehydrogenation and cysteine conjugation) were considered the prominent metabolites in humans. Iruplinalkib-related compounds were found to be covalently bound to proteins, accounting for 7.70% in plasma and 17.96% in feces, which suggested chemically reactive metabolites were formed. There were no serious adverse events observed in the study.

Conclusions: Iruplinalkib was widely metabolized and excreted mainly through feces in humans. Unchanged iruplinalkib, cysteine conjugates and covalent protein binding products were the main drug-related compounds in circulation. Iruplinalkib was well tolerated at the study dose.

Trial Registration: The trial is registered at ClinicalTrials.gov (Identifier:

Anonymized).

Keywords: ALK inhibitors, covalent protein binding, iruplinalkib, mass balance, metabolite profiling, NSCLC

1. Introduction

Oncogenic driver mutations have been identified as new targets for non-small cell lung cancer (NSCLC) treatment[1,2]. Tyrosine kinase inhibitors (TKIs) that target anaplastic lymphoma kinase (ALK) have shown significant superiority over conventional pemetrexed-plus-platinum chemotherapy for the treatment of ALK-positive NSCLC[3,4]. Crizotinib was the first ALK inhibitor approved by the Food and Drug Administration (FDA) for patients with ALK-positive NSCLC. However, acquired resistance and the incidence of brain metastases limit the long-term use of crizotinib in the clinic. Crizotinib remains the first-line treatment for ALK-rearrangement NSCLC, while second-generation ALK inhibitors which overcome crizotinib-resistance, are preferred in both the latest NCCN and CSCO guidelines. The development of novel ALK inhibitors that could improve blood–brain barrier penetration and activity against multiple crizotinib-resistant mutations has great prospects[1,5].

Iruplinalkib (WX-0593) is a novel, highly selective ALK inhibitor that inhibits the activity of both wild-type and resistant mutants of ALK in vitro[6], and shows superior antitumor efficacy in patients with ALK-positive crizotinib-resistant NSCLC and brain metastases[7]. To date, the absorption, distribution, metabolism and excretion (ADME) characteristics of iruplinalkib have not been reported. Human radiolabeled mass balance studies are a feasible method to obtain information on the ADME of investigational drugs[8-10] and are also considered the gold standard for elucidating drug metabolic and excretion characteristics[9,11]. Considering that covalent protein binding is one of the most important characteristics of some TKIs[12], the covalent protein binding characteristics of iruplinalkib were also investigated.

Despite the high efficacy in NSCLC, the adverse effects (AEs) of iruplinalkib during long-term drug treatment should also be noted [7,13]. As reported in the multicenter phase II study (INTELLECT) of iruplinalkib, the most common treatment-related adverse events (TRAEs) of iruplinalkib were increased aspartate aminotransferase (AST) (63/146 [43.2%]) and increased alanine aminotransferase (ALT) (54/146 [37.0%]), indicating that liver injury developed[7]. However, the mechanism behind the AEs is still unclear. Investigating the drug ADME characteristics is essential for elucidating the metabolic pathway and also helping to explain the potential AEs of investigational new drugs from a drug metabolism and pharmacokinetics (DMPK) perspective[14,15].

In this study, a single oral dose of 120 mg/3.7 MBq (100 μCi) [^{14}C]iruplinalkib was administered to six male subjects to clarify the pharmacokinetics, metabolism, covalent protein binding and excretion of iruplinalkib. The potential relationship between the metabolism of iruplinalkib and the AEs during long-term drug treatment was also discussed.

2. Subjects and Methods

2.1 Study design

This was a single-center, nonrandomized, open-label, phase I study (Anonymized) conducted at the Anonymized (Suzhou, China). The subjects received a single oral dose of 120 mg/3.7 MBq [^{14}C]iruplinalkib on the first day of the trial after fasting for at least 10 h and water prohibition for 1 h before administration. Blood and plasma samples, and all urine and feces were collected at specified time points or intervals after drug administration. Sample collection was continued until the cumulative radioactivity exceeded 80% of the administered dose in urine and

feces, the radioactivity in excreta (urine + feces) was less than 1% of the administered dose over a 24-hour period on two consecutive sample collection days, and the plasma radioactivity was less than three times the radioactive background.

The study was approved by the Medical Ethics Committee of the Anonymized and conducted in accordance with the ICH GCP guidelines. All subjects provided written informed consent.

2.2 Subjects

Healthy male subjects aged 18-45 years with a body mass index (BMI) of 19.0-26.0 kg/m^2 were eligible for this study. Health status was determined by routine examinations, including physical examinations, vital signs, clinical laboratory tests, X-rays and 12-lead electrocardiograms. Subjects were excluded if they met any of the following exclusion criteria: a history of cardiovascular, respiratory, renal, hepatic, gastrointestinal disease or major surgery; participation in any other clinical trials in the past 3 months; the use of prescribed or nonprescribed concomitant medications within 14 days of the study; drug or alcohol abuse or smoking addiction. The detailed eligibility criteria are shown in Supplementary Material 1.

2.3 Study medication

Iruplinalkib was synthesized by Qilu Pharmaceutical Co., Ltd. (Shandong, China) and radiolabeled by WuXi AppTec Co., Ltd. (Wuxi, China). The radioactive and chemical purities of [^{14}C]iruplinalkib were 99.53% and 99.28%, respectively. The [^{14}C]iruplinalkib was diluted with an approximate amount of unlabeled iruplinalkib with a target dose of 120 mg/3.7 MBq. The formulation was stored at -20°C, and was removed from the refrigerator to rise to room temperature before use.

2.4 Sample collection

Blood samples (10 mL) were collected into K_2-EDTA anticoagulant tubes at 0 h

(predose), 0.25 h, 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 24 h, 48 h, 72 h, 120 h, 168 h and 216 h after dosing. All blood samples were centrifuged at 2000 ×g for 10 min at 4 °C to obtain supernatants as plasma for radioactivity measurement and liquid chromatography tandem mass spectrometry (LC–MS/MS) analysis, except for whole blood aliquots (2 mL) which were collected at 0 h before dosing and 1 h, 4 h, 8 h, 24 h, 72 h after dosing for radioactivity measurement. Additional blood samples (10 mL) were collected at 1 h, 4 h, 8 h, 24 h, 48 h and 72 h after dosing to isolate plasma for radioactivity measurement and metabolite profiling. Whole blood aliquots and plasma samples were stored at -20 °C or below until analysis.

Urine samples were collected at the following time intervals: -24−0 h (predose), 0−4 h, 4−8 h, 8−12 h, 12−24 h, 24−48 h, 48−72 h, 72−96 h, 96−120 h, 120−144 h, 144−168 h, 168−192 h, 192−216 h, 216−240 h, 240−264 h, 264−288 h, and 288−312 h post dose. After each collection period, the urine samples were mixed thoroughly and weighed. Fecal samples were collected at the following time intervals: -24−0 h (predose), 0−24 h, 24−48 h, 48−72 h, 72−96 h, 96−120 h, 120−144 h, 144−168 h, 168−192 h, 192−216 h, 216−240 h, 240−264 h, 264−288 h, and 288−312 h post dose. All samples were kept at -20 °C or below until analysis.

2.5 Bioanalytical analysis

2.5.1 Pharmacokinetics and mass balance analysis

Blood, plasma, urine and fecal samples were analyzed for total radioactivity (TRA) using a liquid scintillation counter (LSC) (PerkinElmer, Waltham, MA, USA). The radioactivity of the plasma (0.2~0.8 g) and urine (1 g) samples at each collection time point or interval was directly analyzed by LSC. Blood samples (0.5 g) and fecal homogenate (0.3 g) were combusted using a biological oxidizer (Taicang Hualida Laboratory Equipment Co., Ltd) before LSC. Radioactive counts were converted into

the concentration of drug-related components in accordance with the specific activity of the dose, and normalized to total radioactivity collected per interval accounting for total weight.

Unchanged iruplinalkib in human plasma was determined by a validated LC−MS/MS method. A stable isotope-labeled iruplinalkib was used as the internal standard (IS). Protein precipitation for plasma sample preparation was performed using methanol (1:3, v/v). After vortexing and centrifugation, the supernatant was separated on a C18 column (100×2.1 mm, 3.5 μm) with gradient elution. The mobile phase consisted of 2 mM ammonium acetate aqueous solution containing 0.1% formic acid and methanol at a flow rate of 0.8 mL/min. Detection was performed in multiple reaction monitoring (MRM) mode on an LC−MS/MS system (AB SCIEX, CA, USA). The MS/MS transitions used for monitoring were m/z 569.3 to 503.3 for iruplinalkib and m/z 575.3 to 506.4 for the IS. The calibration range for iruplinalkib in human plasma was 2 to 800 ng/mL. A summary of the method validation for the determination of iruplinalkib in human plasma is shown in Supplementary Material 2.

The plasma TRA and iruplinalkib concentration data were used to calculate the pharmacokinetic parameters, including the maximum concentration (C_{max}), time to reach C_{max} (T_{max}), area under the concentration-time curve (AUC) from time zero to the time of the last quantifiable concentration (AUC_{0-t}), AUC from time zero to infinity ($AUC_{0-\infty}$), elimination half-life time ($t_{1/2}$), and the mean retention time (MRT_{0-t}) from time zero to the time of the last quantifiable concentration. The mass balance of iruplinalkib was assessed by the cumulative percentages of radioactivity (% dose) recovered from urine and feces.

2.5.2 Metabolite profiling and identification

A Hamilton “AUC pool” plasma sample (0−72 h) was obtained by pooling

plasma at each time point of a volume proportional to the time interval for each subject [16-18]. Three volumes (v/v) of acetonitrile were added to the pooled plasma sample. After vortexing, sonicating and centrifugation, the supernatant was removed and the pellet was washed twice with methanol. The combined supernatants were evaporated to dryness under a stream of nitrogen. The residues were suspended in methanol–water (1:1, v/v), assayed for extraction recovery and analyzed for metabolite profiling.

For each subject, urine samples (0−120 h) were pooled using an equal percentage by volume according to the time interval. Pooled urine samples were centrifuged to obtain the supernatant for direct analysis. Fecal homogenates (0–216 h) were pooled at the same weight percentages across collection intervals for each subject. The pooled fecal homogenate samples were successively prepared with the same extraction procedure as the plasma samples. The post-extracted solid (PES) from human fecal samples (0−216 h) after extraction was further hydrolyzed using 1 mol/L HCl and NaOH solutions, respectively, for 20 h at 90°C to obtain the hydrolyzed PES. The hydrolyzed PES was then prepared with the same extraction procedure as plasma samples.

The prepared plasma, urine and fecal samples were subjected to a high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan) with radio-assay. Chromatographic separation was achieved on a C18 column (150×4.6 mm I.D.) at 40℃. The mobile phase consisted of 0.4% formic acid (pH adjusted to 3.2 with ammonia) (A) and methanol (B). The flow rate was set at 0.7 mL/min. The gradient elution was as follows: 0% B, 0–3 min; 0–30% B, 3–20 min, 30–50% B, 20–60 min; 50−100% B, 60−65 min; 100% B, 70−72 min; and 0% B, 72−87 min (HPLC method 1). Due to the coelution of unchanged drug and metabolite M3b, 20 mM

ammonium acetate (pH adjusted to 6.7 with acetic acid) (A) and methanol (B) were selected for secondary separation. The new gradient elution was adjusted as 0% B, 0–3 min; 0–55% B, 3–20 min, 55% B, 20–55 min; 55−100% B, 65−70 min; 100% B, 70−72 min; and 0% B, 72−87 min to obtain the relative amount of unchanged drug and metabolite M3b (HPLC method 2). Fractions of chromatography effluents were collected by time (15 sec/fraction) to microplates. The radioactivity in each fraction was determined by a microplate counter system (PerkinElmer, MA, USA). HPLC radio chromatograms were reconstructed using ARC Convert and Evaluation software (version 3.0, AIM Research Company).

After separation by the HPLC system, iruplinalkib and its metabolites were detected by an LC–MS/MS system (Thermo, MA, USA) to obtain accurate mass data for metabolite identification. Mass spectrometry was performed in electrospray ionization (ESI) mode. The ion spray voltage was 3.8 kV. The ion source temperature was set as 320°C. The sheath gas and aux gas were 55 and 15 units, respectively.

2.6 Safety assessment

The safety of [^{14}C]iruplinalkib was evaluated by physical examinations, vital signs, clinical laboratory tests (hematology, serum chemistry, urinalysis and stool analysis) and 12-lead electrocardiograms. AEs were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) grading system (version 5.0).

2.7 Statistical analysis

Statistical analysis was performed on all subjects who were administered with [^{14}C]iruplinalkib and completed the study. Pharmacokinetic parameters were calculated using plasma TRA (analyzed by LSC) and iruplinalkib (analyzed by LC−MS/MS) concentrations, including C_{max}, T_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, $t_{1/2}$ and $MRT_{0\text{-}t}$

using Phoenix WinNonlin (version 7.0, Pharsight Corp.). One gram of plasma was equivalent to 1 mL in the calculation of C_{max} and AUC of TRA. Safety outcomes were presented using descriptive statistics.

3. Results

3.1 Subjects

A total of 54 healthy Chinese male subjects participated in the screening process, and six were successfully enrolled in the study. All of them were administered according to the protocol and completed sample collection and safety evaluation. The subjects had an average age of 26.3 years (range 20−32 years), a mean weight of 61.4 kg (range 53.0−75.5 kg), and a mean BMI of 21.5 kg/m^2 (range 19.3−23.7 kg/m^2). Demographic characteristics are summarized in **Table 1**.

3.2 Safety and tolerability

There were six AEs related to the drug observed in 4 (66.7%) subjects during the study, including cough (three cases, 50.0%), elevated C-reactive protein (one case, 16.7%), elevated gamma-glutamyl transferase (one case, 16.7%) and fever (one case, 16.7%). All AEs were mild (grade one) and resolved without intervention. No serious AEs or other significant abnormalities occurred in the study. A single oral dose of 120 mg/3.7 MBq [^{14}C]iruplinalkib was safe and well tolerated.

3.3 Pharmacokinetics and blood-to-plasma ratio

After a single oral dose of 120 mg/3.7 MBq [^{14}C]iruplinalkib , the main pharmacokinetic parameters of TRA and iruplinalkib in the subjects are summarized in **Table 2**. Iruplinalkib was absorbed quickly and eliminated slowly from plasma, with a T_{max} of approximately 1.5 h and $t_{1/2}$ of approximately 28.6 h (iruplinalkib) and 31.6 h (total radioactivity), respectively. The AUC_{0-t} of TRA and iruplinalkib in

plasma was 5080 ± 1070 h×ng Eq./mL and 3890 ± 555 h×ng/mL, respectively. The blood-to-plasma ratios of total radioactivity were between 0.617 and 1.16. The concentration-time curves of TRA and iruplinalkib are plotted in **Figure 1**.

3.4 Mass balance

The percentages of radioactivity recovered in urine and feces within 312 h after dosing are shown in **Table 3**, and the cumulative radioactivity excretion rates are presented in **Figure 2**. The total recovery of radioactivity from urine and feces accounted for 88.85 ± 2.01% of the administered dose. The radioactivity was excreted mainly through feces, accounting for 68.63 ± 4.01% of the administered dose. Only 20.23 ± 2.76% of the radioactivity was excreted from the urine. Most of the administered radioactivity was excreted within the first 168 h after dosing, representing approximately 86.4% of the administered dose.

3.5 Metabolite profiling and identification

3.5.1 Metabolite profiling

Metabolite profiling in plasma The representative radio-chromatogram of pooled plasma is shown in **Figure 3**. The parent drug was the major radioactive component in plasma, accounting for 76.46% of TRA exposure. The prominent metabolite, M7, contributed approximately 5.81% of the plasma TRA exposure. Other identified trace radioactive metabolites accounted for 0.16% to 0.96% of TRA exposure. The unidentified radioactive peak exposure was lower than 1.51% of the plasma TRA.

Metabolite profiling in urine and feces The representative radio-chromatogram of pooled urine and feces are shown in **Figure 3**. The parent drug in urine and feces accounted for 18.62% and 21.70% of the administered dose, respectively. Metabolite M7 was detected as the primary metabolite in the excreta, accounting for 17.33% and 0.05% of the dose in feces and urine, respectively. Other identified metabolites in

feces included M3b, M11 and M12, accounting for 3.96%, 1.00% and 1.36% of the dose, respectively. The other trace radioactive metabolites detected in excreta accounted for 0.07% to 0.69% of the dose. The unidentified radioactive peak in urine and feces was below 0.98% of the dose.

Non-extractable portion The non-extractable portion accounted for 7.70% of the TRA in plasma, and 17.96% of the administered dose in feces, indicating the covalent binding of iruplinalkib-related components and proteins. The identification of the non-extractable portion is shown in section 3.5.3.

3.5.2 Metabolite identification

Seventeen metabolites of iruplinalkib were identified. With integration of all radioactive peaks, the radioactivity distribution of iruplinalkib and its metabolites expressed as %AUC in plasma and %dose in urine and feces are summarized in **Table 4**. M3b (demethylation), M7 (cysteine conjugation), M11 (oxidative dehydrogenation and cysteine conjugation of M3b) and M12 (oxidative dehydrogenation and cysteine conjugation) were considered the prominent metabolites of iruplinalkib in humans. The mass spectral fragmentation and structures of iruplinalkib and its prominent metabolites are listed in **Table 5**, and the detailed mass spectral data and proposed characteristic fragmentations of iruplinalkib and all the identified metabolites are shown in Supplementary Material 3. The proposed metabolic pathways of iruplinalkib mainly included demethylation, oxidation and cysteine conjugation (**Figure 4**).

3.5.3 Covalent protein binding fraction identification

The main drug-related components of the post-extracted solid hydrolyzed by HCl and NaOH solutions was M7 (cysteine conjugate of iruplinalkib), accounting for 60.71% and 65.48%, respectively. The other components identified from the PES were parent drug (P), P+S-4H and M2, of which the parent drug and M2 were proved

as the secondary metabolites of M7 under acidic and alkaline conditions, respectively. The radio-chromatograms of the hydrolyzed products from PES, and the mass spectra of the major hydrolyzed products are shown in **Figure 5**. The detailed mass spectral data and proposed characteristic fragmentations of the hydrolyzed products from PES are shown in Supplementary Material 3.

4. Discussion

Iruplinalkib is a novel ALK inhibitor that has the advantage of overcoming the acquired crizotinib resistance and the incidence of brain metastases, which makes it a treatment option with wide prospects for clinical application[13].

In this study, a single oral dose of 120 mg/3.7 MBq [^{14}C]iruplinalkib was administered to six healthy subjects to study the ADME of the drug. The dose of the medicine was carefully calculated. The 120 mg of iruplinalkib was within the expected clinical dose range, and the radiation dose of 3.7 MBq (100 μCi) was a conventional dose in human mass balance studies. The dosimetry for subjects who received 120 mg/3.7 MBq [^{14}C]iruplinalkib was estimated to be approximately 0.0408 mSv, indicating a low risk of individual exposure according to the International Commission on Radiological Protection (ICRP). The dosimetry for all organs, including the lens of the eyes, blood-forming organs and gonads was also far below FDA constraints[19].

The present study demonstrated that iruplinalkib was absorbed quickly and slowly eliminated from plasma with a median T_{max} of 1.5 h and an average $t_{1/2}$ of approximately 28.6 h (iruplinalkib) and 31.6 h (total radioactivity). The exposure (AUC_{0-t}) of the total radioactivity was higher than that of unchanged iruplinalkib in plasma, indicating that there were some circulating metabolites in plasma. The blood-

to-plasma ratios of total radioactivity were between 0.617 and 1.16, indicating that iruplinalkib and the metabolites were not significantly bound to blood cells. Urine excretion accounted for 20.23% of the administered dose, indicating that at least 20.23% of the drug was absorbed. Fecal excretion accounted for 68.63% of the administered dose, which included both the unabsorbed dose and the absorbed dose excreted through bile. Since iruplinalkib was a compound with high solubility and permeability, it is speculated that its bioavailability was high, and the dose excreted though feces was mainly from bile excretion after drug absorption. The overall recovery of iruplinalkib in feces and urine was 88.85 ± 2.01% within 312 h, which indicated a general mass balance. The slow elimination of iruplinalkib in plasma and excreta may also be partly related to the covalent protein binding of iruplinalkib-related components in vivo.

Seventeen metabolites of iruplinalkib were identified, and cysteine conjugate products were demonstrated the most abundant metabolites of iruplinalkib. Iruplinalkib-related components were found to be covalently bound to proteins, accounting for 7.70% in plasma and 17.96% in feces, which suggested that chemically reactive metabolites (CRMs) were formed. CRMs are intermediates produced by the metabolic bioactivation of some drugs, which easily covalently bind to sulfhydryl-rich molecules (such as glutathione and cysteine) or proteins due to their electrophilicity. It is now widely accepted that CRMs play an important role in drug-induced liver injury (DILI) [8,20]. As thioether adducts and protein adducts are biomarkers for the exposure of CRMs in vivo, the detection of iruplinalkib-related thioether adducts (cysteine conjugates) and protein adducts (non-extractable portion) in the present study reflects the formation of CRMs. Currently, the mechanism of the hepatotoxicity of CRMs is still not fully understood, but it is thought to begin with the

depletion of sulfhydryl-rich molecules, and therefore to exhibit dose dependence[20]. Given the reserves of glutathione and cysteine in the liver, the hepatotoxicity caused by CRMs may not occur with a single dose of iruplinalkib, but in long-term drug treatment, which is consistent with the safety results of the present and previous studies. However, due to the limitation of the study, the drug-related CRMs production and covalent protein binding at multiple doses remains unclear. In addition, how many doses of iruplinalkib leads to the depletion glutathione and cysteine in the liver needs further investigation. Supplementation with the FDA-approved sulfhydryl antioxidant N-acetylcysteine (NAC) may theoretically help mitigate the DILI caused by CRMs, but its clinical efficacy for the investigational drug still needs future investigation[21].

5. Conclusions

In summary, we report here for the first time the pharmacokinetics, metabolism, covalent protein binding and excretion of iruplinalkib, a novel ALK inhibitor, in humans. Iruplinalkib was well absorbed and mainly excreted through feces. Seventeen metabolites of iruplinalkib were identified, and cysteine conjugate products were the most abundant. CRMs were suggested to be formed during the drug metabolism and then to covalently bind to free cysteine molecules and proteins. Iruplinalkib was well tolerated at the study dose. The present study provides insights into iruplinalkib from a DMPK perspective.

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Declaration of interest

M Ge, H Li and S Zheng are employees of Qilu Pharmaceutical Co., Ltd. All the other authors declare no conflicts of interest. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Author contributions

L Miao and H Zhang were involved in the conception and study design; Y Bian, S Ma, M Ge, H Li, S Zheng, Z Gu, H Feng, Z Yu, C Huang and H Zhang conducted the study; Y Bian, S Ma, Q Yao, T Hu and Z Yu conducted data analyses; Y Bian, S Ma, L Zhao and L Miao were responsible for writing the manuscript. All authors reviewed and approved the manuscript.

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Data availability statement

The data that support the findings of this study are available from the correspondence author upon reasonable request.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

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Table legends

Table 1 Baseline demographics of the subjects.

Table 2 Pharmacokinetic parameters of total radioactivity and iruplinalkib in plasma following a single oral dose of 120 mg/3.7 MBq [^{14}C]iruplinalkib (n=6).

Table 3 Recovered radioactivity in excreta after a single oral dose of 120mg/3.7 MBq [^{14}C]iruplinalkib within 312 h in subjects (n=6).

Table 4 Mean radioactivity distribution of [^{14}C]iruplinalkib and its metabolites expressed as %AUC in plasma, %dose in urine and feces.

Table 5 Mass spectral fragmentation and structures of iruplinalkib and its prominent metabolites.

Figure legends

Fig.1 Plasma concentration-time curves of iruplinalkib and total radioactivity following a single oral dose of 120 mg/3.7 MBq [^{14}C]iruplinalkib given to six healthy subjects (mean+SD).

Fig.2 The cumulative excretion rates after a single oral dose of 120 mg/3.7 MBq [^{14}C]iruplinalkib in six healthy subjects (mean+SD).

Fig.3 Representative radio-chromatograms of iruplinalkib and metabolites in pooled human plasma (0-72 h), urine (0-120 h) and feces (0-216 h).

Fig.4 Proposed metabolic pathways of iruplinalkib in humans.

Fig.5 Radio-chromatograms of PES and M7 hydrolysates by HCl and KOH (A), and the mass spectra of the major hydrolyzed products (B) (Detailed mass spectral data and proposed fragmental pathways shown in Supplementary Material 3).

Tables

Table 1 Baseline demographics of the subjects

Characteristics	Value
Age (years)	
Mean (SD)	26.3 (4.27)
Median (Min, Max)	27.0 (20, 32)
Sex, N (%)	
Male	6 (100)
Race, N (%)	
Han	6 (100)
Height (cm)	
Mean (SD)	168.8 (9.19)
Median (Min, Max)	169.3 (153.0, 178.5)
Weight (kg)	
Mean (SD)	61.4 (8.0)
Median (Min, Max)	60.8 (53.0, 75.5)
BMI (kg/m^2)	
Mean (SD)	21.5 (1.68)
Median (Min, Max)	21.9 (19.3, 23.7)

Table 2 Pharmacokinetic parameters of total radioactivity and iruplinalkib in plasma following a single oral dose of 120 mg/3.7 MBq [^{14}C]iruplinalkib (n=6)

Parameter	Total radioactivity	Iruplinalkib
T_{max} (h)	1.50 (1.00, 2.00)	1.50 (1.00, 1.50)
C_{max}(ng Eq./mL or ng/mL)	333 ± 75.0	276 ± 63.7
AUC_{0-t} (h×ng Eq./mL or h×ng/mL)	5080 ± 1070	3890 ± 555
$AUC_{0-\infty}$ (h×ng Eq./mL or h×ng/mL)	6210 ± 1130	3990 ± 547
$t_{1/2}$ (h)	31.6 ± 7.61	28.6 ± 6.44
MRT_{0-t}（h）	19.5 ± 1.94	22.5 ± 3.23

Data was presented as mean ± SD or median (min, max) as appropriate.

Table 3 Recovered radioactivity in excreta after a single oral dose of 120mg/3.7 MBq [^{14}C]iruplinalkib within 312 h in subjects (n=6)

Subject	Recovery Radioactivity (%dose)		
	Urine	**Feces**	**Total**
1001[a]	15.79	74.29	90.08
1002[a]	23.98	63.36	87.34
1003	21.70	64.88	86.58
1004	20.56	69.81	90.37
1005	20.54	70.89	91.43
1006	18.79	68.52	87.31
Mean ± SD	20.23 ± 2.76	68.63 ± 4.01	88.85 ± 2.01

[a]: Subject 1001 and 1002 collected urine and fecal samples from 0~216 h, while the rest of the subjects collected excreta samples from 0~312 h.

Table 4 Mean radioactivity distribution of [^{14}C]iruplinalkib and its metabolites expressed as %AUC in plasma, %dose in urine and feces

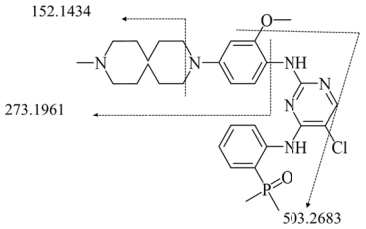
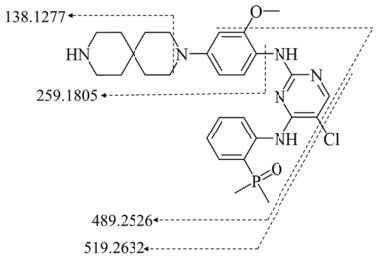
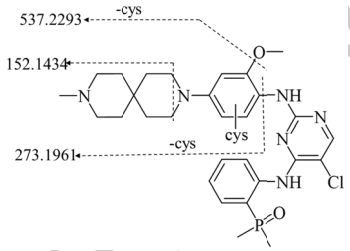
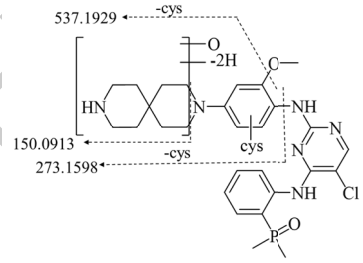
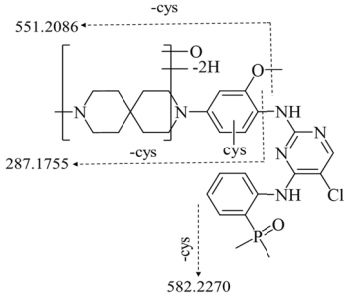
Metabolites	Structural modification	Plasma %AUC	Urine %Dose	Feces %Dose
Iruplinalkib	unchanged drug	76.46 ± 7.66	18.62 ± 2.28	21.70 ± 6.82
M3b*	demethylation	0.16 ± 0.38	+	3.96 ± 0.64
M7*	cysteine conjugation	5.81 ± 2.58	0.05 ± 0.08	17.33 ± 2.65
M11*	demethylation, mono-oxidation, cysteine conjugation	ND	+	1.00 ± 0.32
M12*	mono-oxidation, cysteine conjugation	ND	+	1.36 ± 0.36
M1a	hydrolyzation, mono-oxidation	+	ND	+
M1b	hydrolyzation, mono-oxidation	0.71 ± 0.87	+	ND
M1c	hydrolyzation, mono-oxidation	+	0.69 ± 0.32	+
M2	hydrolyzation	+	+	0.45 ± 0.08
M3a	demethylation	ND	+	+
M4a	mono-oxidation	ND	ND	0.53± 0.14
M4b	mono-oxidation	0.96 ± 1.23	0.31 ± 0.23	+
M5a	di-oxidation	ND	ND	+
M5b	di-oxidation	ND	+	0.07± 0.06
M6	+$C_2H_2O_2S$	ND	ND	+
M8	di-oxidation, dehydrogenation	+	+	0.49 ± 0.16
M9	demethylation, mono-oxidation, dehydrogenation	0.57 ± 0.72	+	+
M10	mono-oxidation, dehydrogenation	+	+	+
	PES	7.70	NA	17.96

* represents the proposed prominent metabolites of iruplinalkib

ND: not detected; NA: no data available; +: only mass spectrum detected; PES: post-extracted solids

Table 5 Mass spectral fragmentation and structures of iruplinalkib and its prominent metabolites

(Other identified metabolites and the detailed mass spectral data and proposed fragmental pathways are shown in Supplementary Material 3)

Metabolites	Structures and theoretical fragment ions	t_R (min)	$[M+H]^+$ (Da)	Characteristic fragment ions
Iruplinalkib		43.1-43.6	569.2552	534.2859, 503.2679, 273.1962, 255.0455, 152.1435
M3b		43.1-43.6	555.2394	520.2703, 489.2531, 259.1812, 255.0450, 138.1280
M7		49.9-50.9	688.2591	568.2475, 553.2255, 537.2297, 303.1533, 255.0454, 273.1963, 152.1434
M11		64.6-64.9	688.2220	568.2105, 537.1925, 303.1155, 273.1594, 255.0447, 150.0916
M12		65.9-66.1	702.2382	582.2258, 551.2077, 317.1310, 287.1748, 255.0446

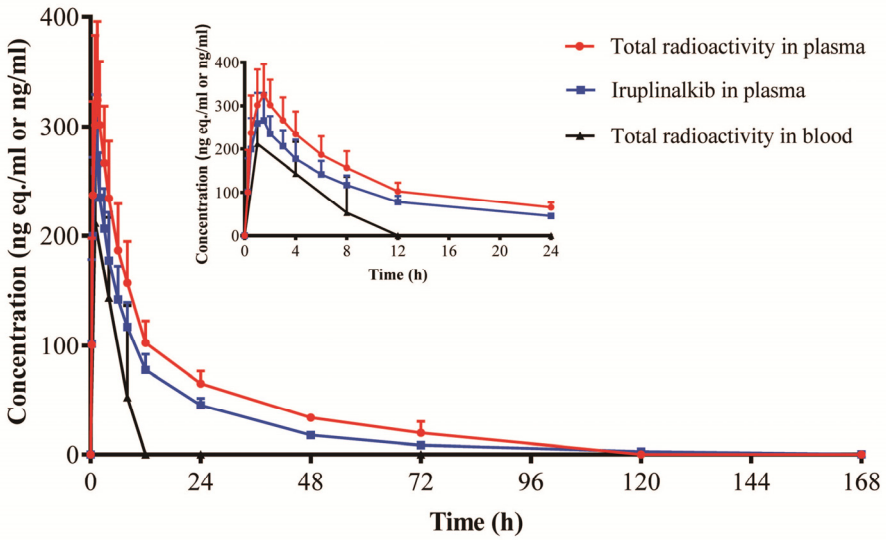


Figure 1

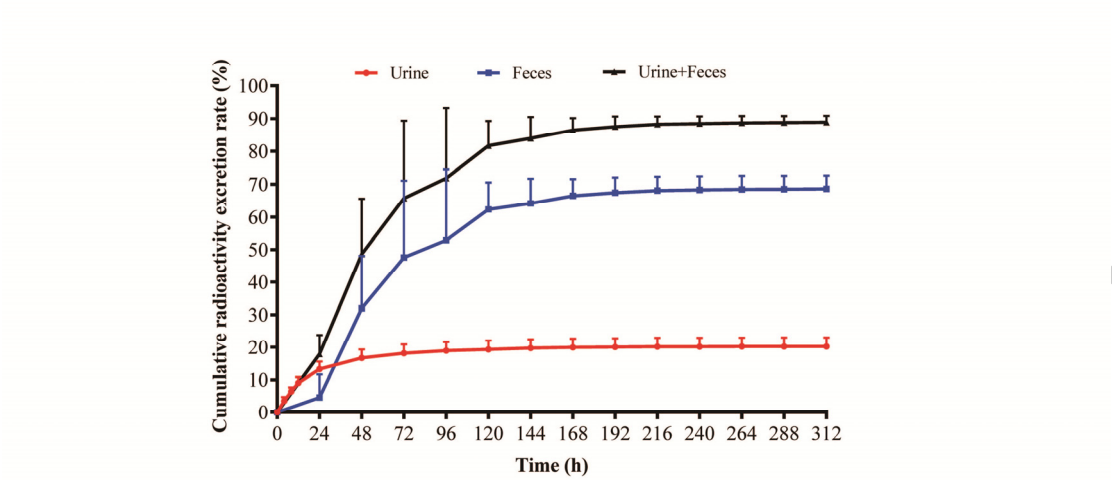


Figure 2

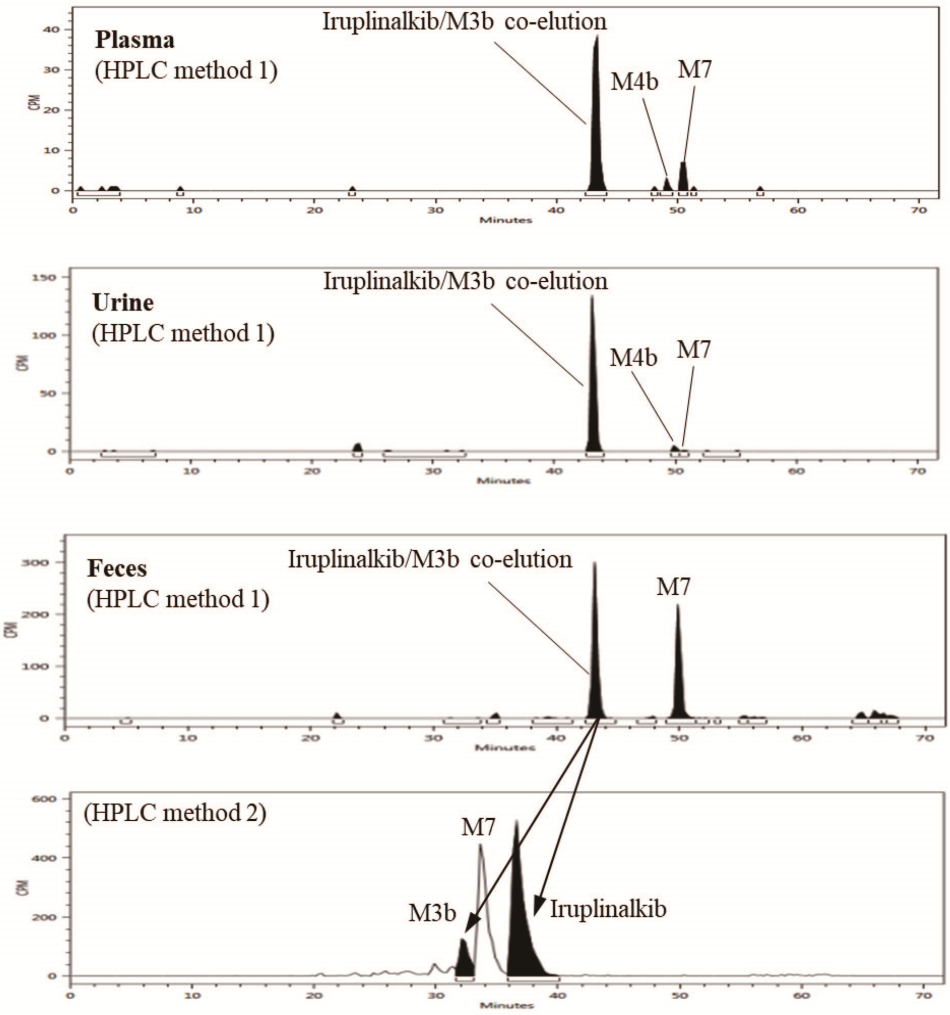
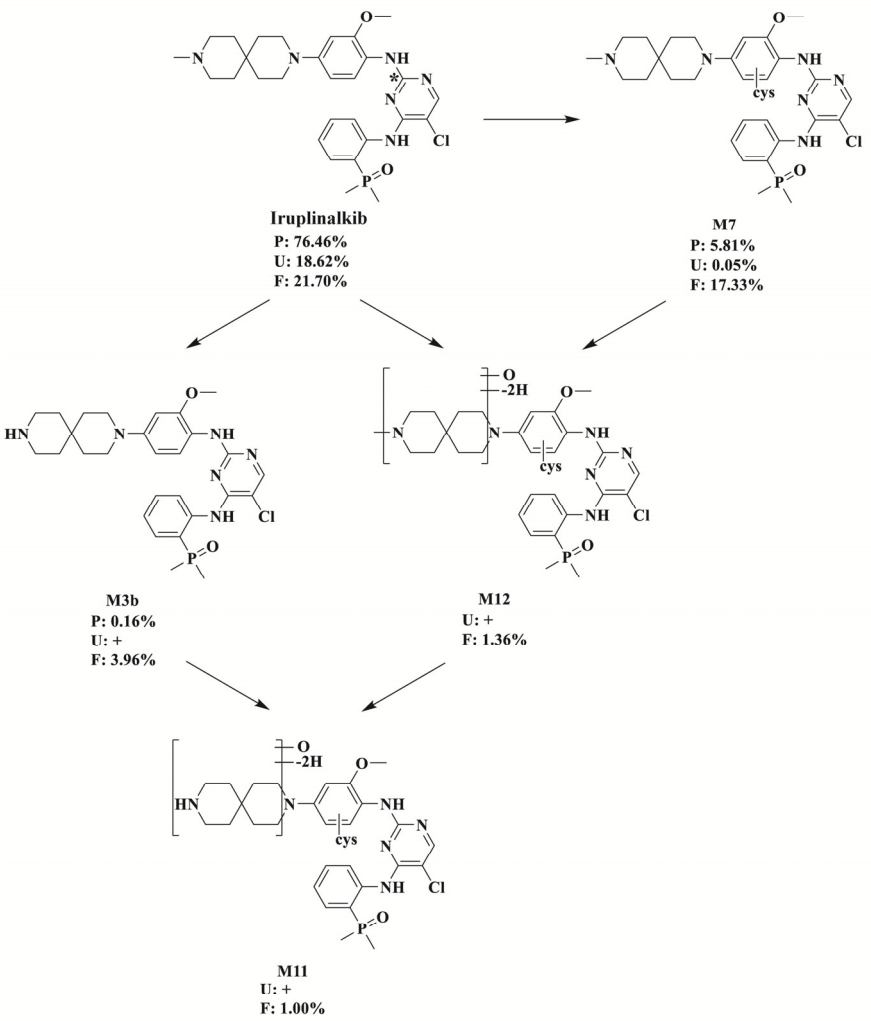


Figure 3



* represented the position of Carbon-14

Figure 4

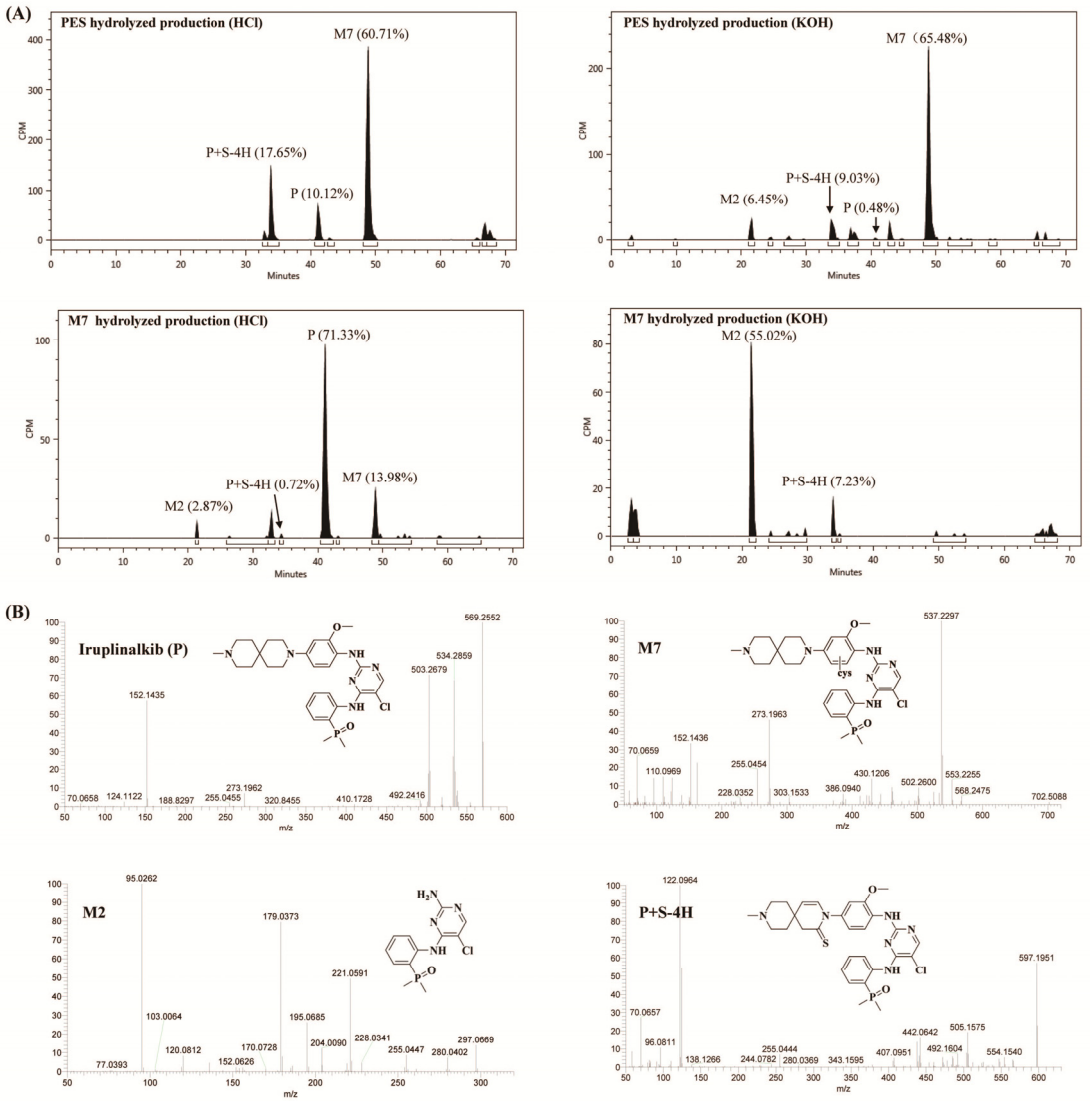


Figure 5

Supplementary Material 1

Eligibility Criteria

Inclusion Criteria:

- 18-45 years old (inclusive) healthy male volunteers;
- Body mass index 19.0-26.0 kg/m^2 (inclusive) and body weight ≥50 kg;
- The volunteer has fully given informed consent to the study and voluntarily signed the informed consent form prior to trial.

Exclusion Criteria:

- Physical examination, vital signs, laboratory tests (routine blood test, urinalysis, stool + occult blood test, blood biochemistry, blood coagulation, and thyroid function), ophthalmic examination (slit lamp exam, intraocular pressure, and fundus imaging), 12-lead electrocardiogram, chest X-ray (posteroanterior view), and abdominal ultrasound results that are determined abnormal and clinically significant by the investigator during screening;
- Positive for HBsAg, HBeAg, HCV antibody, HIV antibody, or syphilis antibody;
- Has taken any prescribed medication and herbal medicine within 1 month prior to dosing, or has taken over-the-counter medications or food supplements (e.g. Vitamins or calcium) within 2 months prior to dosing;
- Has participated in clinical trials of any other drug within 3 months prior to dosing;

- Previous heart disease or family history of heart disease, such as organic heart disease, heart failure, myocardial infarction, angina, irreducible arrhythmia, torsades de pointes, ventricular tachycardia, or long QT syndrome;
- Major surgery within 6 months prior to dosing or surgical incision is not completely healed; Major surgery includes but is not limited to surgery with any significant risk of bleeding, prolonged general anesthesia, biopsy or significant traumatic injury;
- Any history of severe clinical illness or disease and conditions that are judged by the investigator to potentially affect the results of the study or disease and history of disease that can reduce treatment adherence (including but not limited to cardiovascular, liver, kidney, gastrointestinal, immune, urinary, blood, endocrine, metabolic, mental, and neurological diseases as well as cancer), especially a history of dysphagia, gastrointestinal ulcers or any gastrointestinal disorders that affect drug absorption;
- Known allergy to two or more substances; Or potential allergy to the test drug or its excipients in the judgment of the investigator, or allergy to any food ingredients, or special requirements for diet and inability to consume a uniform diet;
- Hemorrhoids or perianal disease with regular/current bleeding;
- Habitual constipation, diarrhea, irritable bowel syndrome, or inflammatory bowel disease;
- Consumed over 14 units of alcohol (1 unit = 360 mL beer or 45 mL of liquor with 40% alcohol or 150 mL wine) weekly within 6 months prior to screening or breath alcohol test result of ≥20 mg/dL;

- Consumed more than 5 cigarettes daily within 3 months prior to screening or inability to quit smoking during the study;
- Consumed soft drugs within 3 months prior to dosing or hard drugs within 1 year prior to dosing or positive for urine drug test;
- Habitually consumed grapefruit or grapefruit juice, or excessively consumed tea, coffee, alcohol and/or drinks containing caffeine or alcohol and unable to quit during hospitalization, or frequent strenuous exercise and other factors that affect drug absorption, distribution, metabolism, and excretion;
- Consumed any food or drink rich in xanthine (e.g. Animal organs, chocolate, tea, coffee or coke) within 48 hr prior to dosing, or any grapefruit or products containing grapefruit within 48 hr prior to dosing;
- Donated blood within 3 months prior to dosing or lost >400 mL of blood or received blood transfusion within 8 weeks prior to dosing;
- Plan to conceive during and within 1 year after trial completion, or disagree with taking strict contraceptive measures (by the volunteer and his spouse) during and within 1 year after trial completion;
- Work in conditions with long-term radiation exposure, or had significant radiation exposure (received ≥2 chest/abdominal CT, or ≥3 other x-ray exams) within 1 year of signing the informed consent, or participated in radioactive drug trials, or plan to have radiation exposure within 1 year after trial completion.
- Any other factors which in the judgment of the investigator render the volunteer unsuitable for the trial.