Shionogi Study Title:	A Phase 1, Randomized, Double-Blind, Single-Ascending-Dose, and Food Effect Study to Assess the Safety, Tolerability, Ventricular Repolarization, and Pharmacokinetics of S-648414 in Healthy Adult Study Participants (Part 1); A Phase 1, Randomized, Double-Blind, Multiple-Ascending-Dose Study to Assess the Safety, Tolerability, and Pharmacokinetics of S-648414 and A Drug-Drug Interaction Study with the CYP3A Substrate, Midazolam, in Healthy Adult Study Participants (Part 2); and A Phase 1 Open-Label Study to Assess the Effect of S-648414 on the Pharmacokinetics of Dolutegravir and the Effect of Dolutegravir on the Pharmacokinetics of S-648414 in Healthy Adult Study Participants (Part 3)
Shionogi Study Number:	1908T0911
ClinicalTrials.gov Registration No.	NCT04147715
Study Document	Protocol Amendment 6 June 17, 2020

History of Protocol Amendments

Original	July 30, 2019
• Modifies the dose in Groups G and H in Part 2	
Amendment 1	December 23, 2019
• Adds Part 3 to study the effect of S-648414 on the pharmacokinetics of dolutegravir and the effect of dolutegravir on the PK of S-648414	
Amendment 2	January 27, 2020
• Adjusts the dose for Group H and Group I	
Amendment 3	February 9, 2020
• Adds ophthalmological examinations to Part 3 of the study	
Amendment 4	March 16, 2020
• Adds a second center and region to the study • Change doses in Part 3 of the study	
Amendment 5	May 6, 2020
• Adds text to allow the investigator to choose between outpatient or inpatient status during Days 9 to 13 between Periods 1 and 2 of Part 3	
Amendment 6	June 17, 2020

TITLE PAGE

Protocol Title:

A Phase 1, Randomized, Double-Blind, Single-Ascending-Dose, and Food Effect Study to Assess the Safety, Tolerability, Ventricular Repolarization, and Pharmacokinetics of S-648414 in Healthy Adult Study Participants (Part 1); A Phase 1, Randomized, Double-Blind, Multiple-Ascending-Dose Study to Assess the Safety, Tolerability, and Pharmacokinetics of S-648414 and A Drug-Drug Interaction Study with the CYP3A Substrate, Midazolam, in Healthy Adult Study Participants (Part 2); and A Phase 1 Open-Label Study to Assess the Effect of S-648414 on the Pharmacokinetics of Dolutegravir and the Effect of Dolutegravir on the Pharmacokinetics of S-648414 in Healthy Adult Study Participants (Part 3)

Protocol Number: 1908T0911

Amendment Number: 6

Compound Number: S-648414

Short Title: Phase 1 Study of S-648414

Sponsor Name: Shionogi

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Issued Date: 17 Jun 2020

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PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Date**
Amendment 6	17 Jun 2020
Amendment 5	06 May 2020
Amendment 4	16 Mar 2020
Amendment 3	09 Feb 2020
Amendment 2	27 Jan 2020
Amendment 1	23 Dec 2019
Original Protocol	30 Jul 2019

Amendment 6 (17 Jun 2020)

Overall Rationale for the Amendment:

This amendment allows the investigator to choose between outpatient or inpatient status during Days 9 to 13 between Periods 1 and 2 of Part 3.

A high-level description of the changes and brief scientific rationale are outlined in the following:

Section(s) # and Name(s)	Description of Change	Brief Rationale
1.1 Synopsis, Overall Design, Part 3, Figure 1-4, 4.1 Overall Design, and Table 4-3	Added text to change the outpatient period (Days 9 to 13 between Periods 1 and 2 of Part 3) to be either outpatient or inpatient.	This will allow the investigator to determine if a study participant should remain inpatient or can be outpatient during Days 9 to 13.
1.1 Synopsis, Safety Assessments, Figure 1-4, Figure 1-5, and 8.2.2 Vital Signs	Removed specification of oral body temperature for Part 3.	Any method of temperature measurement is acceptable, axillary temperature is standard of care in Japan.
7.1 Discontinuation of Study Intervention	Allow replacement of study participants who prematurely discontinue during Part 3.	Allows study participants to be replaced during Part 3.

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1. PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title:

A Phase 1, Randomized, Double-Blind, Single-Ascending-Dose, and Food Effect Study to Assess the Safety, Tolerability, Ventricular Repolarization, and Pharmacokinetics of S-648414 in Healthy Adult Study Participants (Part 1); A Phase 1, Randomized, Double-Blind, Multiple-Ascending-Dose Study to Assess the Safety, Tolerability, and Pharmacokinetics of S-648414 and A Drug-Drug Interaction Study with the CYP3A Substrate, Midazolam, in Healthy Adult Study Participants (Part 2); and A Phase 1 Open-Label Study to Assess the Effect of S-648414 on the Pharmacokinetics of Dolutegravir and the Effect of Dolutegravir on the Pharmacokinetics of S-648414 in Healthy Adult Study Participants (Part 3)

Protocol Number: 1908T0911

Compound Number: S-648414

Short Title: Phase 1 Study of S-648414

Rationale:

S-648414 is a novel antiretroviral agent that is being developed as a treatment for human immunodeficiency virus (HIV-1) infection in combination with other antiretroviral agents to fully suppress HIV-1 viremia. Based on a full pre-clinical development program of S-648414, this study is expected to be conducted safely and the minimum and maximum doses tested have been determined based on in-vitro efficacy estimations and animal Good Laboratory Practice (GLP)-compliant toxicology studies. These Phase 1 assessments will describe the safety, tolerability, and pharmacokinetics (PK) of S-648414 following first-in-human dosing, both single- and multiple-ascending dosing (SAD/MAD), food effect, effect on electrocardiogram (ECG) parameters, and effect on the PK of midazolam, a cytochrome P450 3A (CYP3A) probe, as well as the effect of S-648414 on the PK of dolutegravir and the effect of dolutegravir on the PK of S-648414. The study will permit evaluation of dosing and safety in the clinical setting to inform and facilitate studies in individuals living with HIV-1 infection.

Objectives and Endpoints:

Table 1-1 Objectives and Endpoints

Objectives	Endpoints
Primary	
Part 1: • To evaluate the safety and tolerability of S-648414 after administration of a single oral dose of S-648414 in healthy adult study participants **Part 2:** • To evaluate the safety and tolerability of S-648414 after administration of multiple oral doses of S-648414 in healthy adult study participants **Part 3:** • To evaluate the safety and tolerability of S-648414 after administration of multiple oral doses of S-648414 in healthy adult study participants • To evaluate the effect of S-648414 on the PK of dolutegravir in healthy adult study participants • To evaluate the effect of dolutegravir on the PK of S-648414 in healthy adult study participants	**Part 1:** • The number and percentage of clinical and laboratory adverse events in study participants exposed to single dose of S-648414 **Part 2:** • The number and percentage of clinical and laboratory adverse events in study participants exposed to multiple doses of S-648414 **Part 3:** • The number and percentage of clinical and laboratory adverse events in study participants exposed to multiple doses of S-648414 • S-648414 and dolutegravir: C_{max}, T_{max}, C_τ, AUC, and CL/F
Secondary	
Part 1: • To assess the PK of S-648414 after administration of a single oral dose of S-648414 in healthy adult study participants • To assess the effect of a high-fat meal on the PK of S-648414 after administration of a single oral dose of S-648414 in healthy adult study participants • To assess the effect of S-648414 on ECG parameters in healthy adult study participants **Part 2:** • To assess the PK of S-648414 after administration of multiple oral doses of S-648414 in healthy adult study participants • To evaluate the potential of S-648414 for inhibition or induction of the CYP3A enzymes using MDZ as a probe substrate after administration of multiple oral doses of S-648414 in healthy adult study participants **Part 3:** • To evaluate the safety and tolerability after coadministration of dolutegravir with S-648414 in healthy adult study participants	**Parts 1 and 2:** • S-648414: C_{max}, T_{max}, AUC, $t_{1/2,z}$, λ_z, MRT, CL/F, V_z/F, CL_R, and Feu following single oral dosing, multiple dosing and in fasted and fed state • Change from baseline in HR, QTcF, PR and QRS (ΔHR, ΔQTcF, ΔPR and ΔQRS) • Placebo-corrected ΔHR, ΔQTcF, ΔPR and ΔQRS (ΔΔHR, ΔΔQTcF, ΔΔPR and ΔΔQRS) • Categorical outliers for HR, QTcF, PR, QRS • Frequency of treatment-emergent changes for T-wave morphology and U-wave presence **Part 2 only:** • MDZ: C_{max}, T_{max}, AUC, $t_{1/2,z}$, λ_z, and MRT **Part 3 only:** • The number and percentage of clinical and laboratory adverse events in study participants exposed to multiple doses of S-648414 and dolutegravir

AUC = area under the concentration-time curve; C_τ = plasma concentration at the end of the dosing interval τ (24 hours); CL/F = apparent total clearance; CL_R = renal clearance; C_{max} = maximum plasma concentration; CYP3A = cytochrome P450 3A; ECG = electrocardiogram; Feu = fraction of dose excreted in urine; HR = heart rate; MDZ = midazolam; MRT = mean residence time; PK = pharmacokinetics; PR = measure between beginning of P wave until beginning of QRS complex; QRS = combination of the Q, R, and S waves; QTcF = Fridericia's corrected QT; T_{max} = time to maximum plasma concentration; $t_{1/2,z}$ = terminal elimination half-life; λ_z = terminal elimination rate constant; Vz/F = apparent volume of distribution in the terminal elimination phase; Δ = change from baseline; ΔΔ = placebo-corrected change from baseline

Overall Design:

The study is a multicenter, randomized, double-blind (Parts 1 and 2) and open-label (Part 3), placebo-controlled, sequential group study in healthy adult study participants.

The study consists of Part 1 (SAD, food effect, and effects on ECG parameters including concentration-QTc analysis), Part 2 (MAD and drug-drug interaction with the CYP3A substrate), and Part 3 (the effect of S-648414 on the PK of dolutegravir and the effect of dolutegravir on the PK of S-648414). The sequence of steps in Parts 1, 2, and 3 are summarized in Table 1-5.

Part 1:

The study consists of a Screening period (Days −28 to −2), admission to the clinical research unit (CRU, Day −1), single administrations of S-648414 or matching placebo (Day 1), discharge from the CRU (Day 5), and a Follow-up visit (Day 10 ± 2) (see Figure 1-2).

In Groups A, B, D, E, and F, eligible healthy adult study participants will be assigned to 1 of 5 ascending dose groups (10 to 1000 mg) and receive a single oral dose of S-648414 or placebo in a fasted state with 6 study participants per dose group receiving active drug and 2 study participants per dose group receiving placebo. Administration initiates from the lowest dose group, and the next group does not initiate until the Follow-up period has been completed for the previous group except for A-2 (see Table 1-2).

In Group C, 8 study participants will be assigned to S-648414, and 2 study participants will be assigned to placebo. Study participants assigned to the 100-mg group will receive a single oral dose of S-648414 in a fasted state (Group C-1), followed by a single dose of S-648414 in a fed state (high-fat meal) (Group C-2). Each episode of dosing will be separated by a washout period of at least 14 days.

Table 1-2 Part 1 Dose Groups

Group Label	S-648414 Dose	Number of Active Study Participants	Number of Placebo Study Participants
Group A (Group A-1 and A-2)[a]	10 mg	6	2
Group B	30 mg	6	2
Group C (Group C-1 and C-2)	100 mg	8	2
Group D	250 mg	6	2
Group E	500 mg	6	2
Group F	1000 mg	6	2

a Because this is a first-in-human administration, the initial dose group (Group A) is divided into Group A-1 including 2 study participants (1 S-648414 and 1 placebo) and Group A-2 including 6 study participants (5 S-648414 and 1 placebo). Study participants in Group A-2 will receive study intervention (S-648414 or placebo) in a sentinel dosing sequence, ie, only after confirmation that there are no clinical concerns in Group A-1 within 24 hours postdose.

The doses may be changed depending on the results of the safety, tolerability, and PK in the preceding group(s), if necessary. The highest dose will not exceed 1000 mg.

The investigator **and** the sponsor will evaluate the results of the safety and tolerability in the preceding group(s) and decide whether to proceed to the next dose group.

The next dose group will not be conducted if any of the following criteria are met:

- Any study participant experiences a treatment-related serious adverse event (SAE) (ie, treatment-emergent SAE considered related to the study intervention [S-648414 or placebo] by the investigator and sponsor)
- ≥ 50% of study participants experience grade 2 to 4 treatment-related adverse event (AEs)
- ≥ 25% of study participants experience grade 3 or 4 treatment-related AEs

See Section 10.3 (Appendix 3) for definitions of AEs, SAEs, assessment of intensity (including HIV grading system), assessment of causality, and procedures for recording, evaluating, follow-up, and reporting.

Part 2:

Eligible healthy adult study participants are assigned to 1 of 2 dose groups (50 mg in Group G or 30 mg in Group H) and receive multiple oral doses of S-648414 tablet or placebo in a fed state with 8 study participants receiving active drug and 2 study participants receiving placebo per group (see Table 1-3). Administration of the next dose group (Group H) will not initiate until the Follow-up period has been completed for the previous group (Group G).

In Groups G and H, the study consists of a Screening period (Days −28 to −4), admission to the CRU (Day −3), administration of midazolam 5 mg (Day −2 and Day 14)

and multiple administrations of S-648414 or matching placebo (Days 1 to 14), discharge from the CRU (Day 19), and a Follow-up visit (Day 25 ± 2) (see Figure 1-3).

All study participants will receive a single oral dose of midazolam 5 mg alone on Day -2 and coadministered with the S-648414/placebo dose on Day 14. Study participants will receive S-648414/placebo once daily (QD) on Days 1 to 14 (Group G will receive 50 mg and Group H will receive 30 mg).

Table 1-3 Part 2 Dose Groups

Group Label	S-648414 Dose	Number of Active Study Participants	Number of Placebo Study Participants	Length of Treatment (Days)
Group G	50 mg	8	2	14
Group H	30 mg	8	2	14

The doses, mode of administration, frequency (ie, QD or twice daily [BID]), dietary condition (ie, fasted or fed), or length of administration may be changed depending on the results of the safety, tolerability, and PK in the preceding group(s), if necessary.

The investigator **and** the sponsor will evaluate the results of the safety and tolerability in the preceding group(s) and decide whether to proceed to the next dose group.

The next dose group will not be conducted if any of the following criteria are met:

- Any study participant experiences a treatment-related SAE (ie, treatment-emergent SAE considered related to the study intervention [S-648414 or placebo] by the investigator and sponsor)
- ≥ 50% of study participants experience grade 2 to 4 treatment-related AEs
- ≥ 25% of study participants experience grade 3 or 4 treatment-related AEs

See Section 10.3 (Appendix 3) for definitions of AEs, SAEs, assessment of intensity (including HIV grading system), assessment of causality, and procedures for recording, evaluating, follow-up, and reporting.

Part 3:

Part 3 is an open-label, non-randomized, 1-sequence, 3-period study.

A total of 14 healthy adult study participants will be enrolled in Group I of Part 3; an additional 14 healthy adult study participants will be enrolled in Group J of Part 3.

Part 3 will consist of:

- Screening period (Days −28 to −2)
- Period 1 (admission to CRU on Day -1 with continued confinement until Day 8)
- Mandatory 7-day washout between the last dose of study intervention in Period 1 and the first dose of study intervention in Period 2

  - Day 8 of Period 1 (no dose)
  - Days 9 to 13 (5-days, no dose)

    The 5-day period can be inpatient or outpatient and will be decided at the discretion of the investigator.
  - Day 14 of Period 2 (no dose)
- Period 2 (confinement: Days 14 to 21)
- Period 3 (confinement: Days 22 to 29)
- Follow-up visit (7 days after discharge from the CRU of Period 3 [Day 36] ± 2 days)

Study participants will receive:

- Dolutegravir (50 mg orally QD for 7 days) in the fed state during Period 1
- S-648414 alone (100 mg orally QD for 7 days in Group I and 200 mg orally QD for 7 days in Group J) in the fed state during Period 2
- S-648414 (100 mg orally QD for 7 days in Group I and 200 mg orally QD for 7 days in Group J) coadministered with dolutegravir (50 mg orally QD for 7 days) in the fed state during Period 3

See Table 1-4 for the treatment schedule and Figure 1-1 for the study schematic.

Table 1-4 Part 3 Treatment Schedule

Group Label	Number of Study Participants	Period 1[a]	Period 2[a]	Period 3
Group I	14	Dolutegravir 50 mg QD for 7 days	S-648414 100 mg QD for 7 days	S-648414 100 mg + dolutegravir 50 mg QD for 7 days
Group J	14	Dolutegravir 50 mg QD for 7 days	S-648414 200 mg QD for 7 days	S-648414 200 mg + dolutegravir 50 mg QD for 7 days

QD = once daily

a There will be a 7-day washout period between Periods 1 and 2; the 7-day washout period includes the time when the study participant will not receive study intervention on the last day of Period 1 and the first day of Period 2.

The doses, mode of administration, frequency (ie, QD or BID), dietary condition (ie, fasted or fed), or length of administration may be changed, if necessary, by the sponsor depending on the results of the safety, tolerability, and PK in the preceding group(s).

The investigator **and** the sponsor will evaluate the results of the safety and tolerability in the preceding group(s) and decide whether to proceed to the next dose group.

The next dose group will not be conducted if any of the following criteria are met:

- Any study participant experiences a treatment-related SAE (ie, treatment-emergent SAE considered related to S-648414 by the investigator and sponsor)
- ≥ 50% of study participants experience grade 2 to 4 treatment-related AEs
- ≥ 25% of study participants experience grade 3 or 4 treatment-related AEs

See Section 10.3 (Appendix 3) for definitions of AEs, SAEs, assessment of intensity (including HIV grading system), assessment of causality, and procedures for recording, evaluating, follow-up, and reporting.

Inclusion Criteria:

Study participants are eligible to be included in the study only if all of the following criteria apply:

1. Male or female adults ≥ 18 years in USA or ≥ 20 years in Japan to ≤ 55 years of age, at the time of signing the informed consent form (ICF).
   a) Specific to Japan sites: enrollment in Part 3 (Group I and J) will consist of only White or Black or African American race.
2. Capable of giving signed informed consent as described in Section 10.1 (Appendix 1), which includes compliance with the requirements and restrictions listed in the ICF and in this protocol.
3. Body mass index (BMI) ≥ 18.5 to < 32.0 kg/m^2 at the Screening visit.
4. Considered medically healthy as determined by the investigator or subinvestigator (suitably qualified), based on medical history and clinical evaluations including physical examination, clinical laboratory tests, vital sign measurements, and 12-lead ECG at Screening and at upon admission to the CRU and prior to administration of study intervention on Day 1. Clinical evaluations during Screening can be repeated (for confirmatory purposes or if additional information is required to assess study participant's eligibility) at the discretion of the investigator; repeated procedures must be performed within the same Screening window.
5. Female study participants must not be a woman of childbearing potential (see Section 10.4 [Appendix 4]) and must either be postmenopausal (defined as no menses for 12 months without an alternative medical cause; follicle-stimulating hormone [FSH] to be tested for confirmation at Screening) or premenopausal with 1 of the following documented: hysterectomy, tubal ligation, bilateral salpingectomy, or bilateral oophorectomy.
6. Male study participants must agree to use contraception as detailed in Section 10.4 (Appendix 4) during the treatment period and for at least 3 months after the last dose of study intervention.

Exclusion Criteria:

Study participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Considered by the investigator or subinvestigator (suitably qualified) to be ineligible for the study due to a history of or current condition of significant metabolic or endocrine, hepatic, renal, hematological, pulmonary, cardiovascular, gastrointestinal (GI), urological, immunological, neurological, or psychiatric disorders with clinical manifestations.
2. History or presence of cancer in last 5 years except for nonmelanoma skin cancers.
3. Risk factors for:
    a. Torsades de Pointes (eg, heart failure, cardiomyopathy, or family history of Long QT Syndrome or Brugada Syndrome)
    b. Unexplained syncope, sick sinus syndrome, second- or third-degree atrioventricular (AV) block, myocardial infarction, pulmonary congestion, cardiac arrhythmia, angina, prolonged QT interval, or conduction abnormalities
4. History of GI surgery or disease including, but not limited to, gastric band/gastric resection and/or intestinal resection and or duodenal disease (ie, celiac disease) that may result in clinically significant malabsorption (except for an appendectomy).
5. History of hypersensitivity or severe side effects induced by a drug.
6. Any condition requiring medication and/or other treatment, such as dietary restriction and physical therapy.
7. History of significant multiple and/or severe allergic symptoms including food allergy (NOTE: Study participants with seasonal allergies may participate unless they have ongoing symptoms).

Prior/Concomitant Therapy

8. Used drugs or substances known to be inducers or inhibitors of cytochrome P450 enzymes and/or P-glycoprotein within 28 days prior to admission to the CRU.
9. Used prescription or over-the-counter (OTC) drugs, antacids, proton pump inhibitors, H2 antagonists, Chinese herbal medicines, oral cannabidiol, vitamins, minerals, herbal, and dietary supplements within 14 days prior to admission to the CRU.

Caffeine, Alcohol, Recreational Drugs, and Tobacco or Nicotine

10. Refuses to abstain from ingesting caffeine- or xanthine-containing products/medications (eg, coffee, tea, cola drinks, other caffeinated beverages, or chocolate) from 24 hours prior to admission to the CRU or refuses to refrain from consuming such products throughout the study (including Follow-up period).
11. Consumed alcohol or used alcohol-containing products within 72 hours prior to admission to the CRU or refuses to refrain from consuming such products throughout the study (including Follow-up period).

12. History of recreational drug use in the previous 6 months or has a history of problematic alcohol use (defined as study participants who regularly consume excessive amounts of alcohol, defined as > 3 glasses of alcoholic beverages per day (1 glass is approximately equivalent to: beer [284 mL/10 ounces (oz.)], wine [125 mL/4 oz.] or distilled spirits [25 mL/1 oz.]).
13. A positive drug or alcohol screen at the Screening visit or upon admission to the CRU.
14. Used tobacco- or nicotine-containing products (including cigarette, pipe, cigar, chewing, nicotine patch, or nicotine gum) within 6 months prior to admission to the CRU or refuses to refrain from using tobacco- or nicotine-containing products throughout the study (including Follow-up period).

Meals and Dietary Restrictions

15. Consumed grapefruit, grapefruit juice, Seville orange juice, orange juice, apple juice, vegetables from the mustard green family (eg, kale, broccoli, watercress, collard greens, kohlrabi, Brussels sprouts, mustard greens), or charbroiled meats within 7 days prior to admission to the CRU or refuses to refrain from consuming such products throughout the study (including Follow-up period).

Diagnostic Assessments

16. A corrected QT (QTc) interval of > 450 msec for males and > 470 msec for females (Fridericia's method) at the Screening visit or upon admission to the CRU.
17. Systolic blood pressure is outside the range of 90 to 140 mm Hg, diastolic blood pressure is outside the range of 50 to 90 mm Hg, or pulse rate is outside the range of 40 to 100 beats per minute (bpm) or considered ineligible by the investigator or subinvestigator at the Screening visit or upon admission to the CRU.
18. Total bilirubin, alanine aminotransferase (ALT), or aspartate aminotransferase (AST) values are greater than the upper limit of normal (ULN), or estimated glomerular filtration rate (eGFR) < 90 mL/min/1.73 m^2 at Screening or upon admission to the CRU.
19. A positive serological test for untreated syphilis, positive hepatitis B surface antigen, positive hepatitis C virus antibody, or positive human immunodeficiency virus (HIV) antigen/antibody result at the Screening visit.

Prior/Concurrent Clinical Study Experience

20. Participated in any other investigational trials or has been exposed to other investigational drugs within 28 days or 5 half-lives of the previously administered investigational drug (date derived from last study procedure [blood collection or dosing] of previous trial), whichever is longer, prior to admission to the CRU.
21. Previously received S-648414.

Other Exclusions

22. Poor venous access.

23. Donated blood (> 500 mL) or had significant blood loss within 56 days of study admission to the CRU or donated plasma within 7 days prior to until admission to the CRU.
24. Considered inappropriate for participation in the study for any reason by the investigator or subinvestigator.

Number of Study Participants:

Up to 50 healthy adult study participants in Part 1, 20 healthy adult study participants in Part 2, and 28 healthy adult study participants in Part 3.

Study Intervention Groups and Duration:

Test Drug, Dose, and Mode of Administration:

Part 1:

S-648414 tablets 10 mg, 100 mg, or a combination of both in the fasted (Groups A, B, C-1, D, E, and F) or fed state (Group C-2)

Part 2:

S-648414 tablets 10 mg in the fed state (Groups G and H)

Part 3:

S-648414 tablets 100 mg in the fed state (Groups I and J)

Control Drug, Dose, and Mode of Administration:

Matching placebo tablet, oral administration in the fasted or fed state

Other Treatment, Dose, and Mode of Administration:

Part 2:

Midazolam 5 mg (midazolam 2 mg/mL syrup 2.5 mL), oral administration in the fed state

Part 3:

Dolutegravir 50-mg tablet, oral administration in the fed state

Duration of Study Intervention

Part 1:

S-648414 or matching placebo: single dose (1 day)

Part 2:

S-648414 or matching placebo: multiple doses (14 days) in Groups G and H

Midazolam 5 mg on Day -2, S-648414 on Days 1 to 13, and S-648414 and midazolam 5 mg coadministered on Day 14

Part 3:

Multiple doses of dolutegravir once daily during Period 1 (7 days), multiple doses of S-648414 once daily during Period 2 (7 days), and multiple doses of S-648414 and dolutegravir 50 mg coadministered once daily during Period 3 (7 days)

Study Duration for Individual Study Participants (Includes Screening, Treatment and Follow-up Period):

Part 1:

2 to 6 weeks (5 to 9 weeks for study participants in Group C)

Part 2:

4 to 8 weeks

Part 3:

5 to 9 weeks

Data Monitoring Committee:

No independent data monitoring committee will be established for this study.

Prohibited Concomitant Therapy:

Study participants must abstain from taking prescription or OTC drugs (including antacids, proton pump inhibitors, H2 antagonists, Chinese herbal medicines, oral cannabidiol, vitamins, minerals, herbal, and dietary supplements) within 14 days before the start of the first dose of study intervention (S-648414 or placebo) until completion of the Follow-up visit.

In addition, from 28 days prior to admission to the completion of the study (including Follow-up period) the use of drugs or substances known to be inducers or inhibitors of cytochrome P450 enzymes and/or P-glycoprotein are prohibited.

Concomitant therapies and medical procedures are prohibited during the study unless they are essential for the treatment of an AE:

- From 14 days prior to admission to the completion of the study (including Follow-up period): the use of any therapies (prescribed or OTC drugs; antacids; proton pump inhibitors; H2 antagonists; and vitamins, minerals, herbal, and dietary supplements that have therapeutic effects), except for the study intervention (S-648414 or placebo)
- During the study (admission to the completion of the study, including Follow-up period): medical procedures are prohibited unless essential for treatment of an AE.

Pharmacokinetic Assessments:

Drug Concentration Measurements

Blood and urine samples will be collected for analysis of plasma and urine S-648414 concentrations.

Pharmacokinetic Analyses

Based on the plasma and urine concentrations of S-648414, the following PK parameters will be calculated:

Part 1:

Maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), area under the concentration-time curve (AUC), terminal elimination half-life ($t_{1/2,z}$), terminal elimination rate constant (λ_z), mean residence time (MRT), apparent total clearance (CL/F), apparent volume of distribution in the terminal elimination phase (V_z/F), renal clearance (CL_R), and fraction of dose excreted in urine (Feu)

Part 2:

Based on the plasma concentrations of S-648414 and midazolam, the following PK parameters will be calculated:

- S-648414: C_{max}, T_{max}, AUC over the dosing interval τ ($AUC_{0-\tau}$), CL/F, $t_{1/2,z}$, λ_z, V_z/F, Feu over the dosing interval τ ($Feu_{0-\tau,}$), CL_R
- midazolam: C_{max}, T_{max}, AUC from time zero to the time of the last quantifiable concentration after dosing (AUC_{0-last}), AUC extrapolated from time zero to infinity (AUC_{0-inf}), $t_{1/2,z}$, λ_z, and MRT

Part 3:

Based on the plasma concentrations of S-648414 and dolutegravir, the following PK parameters will be calculated:

C_{max}, T_{max}, plasma concentration at the end of dosing interval τ (24 hours) (C_τ), $AUC_{0-\tau}$, and CL/F.

Safety Assessments:

Adverse events will be collected from the time of signing ICF to up to 10 days after the last dose of study intervention or last Follow-up visit.

Additional safety assessments will include physical examinations, clinical laboratory tests (hematology, blood chemistry, and urinalysis), vital signs (systolic and diastolic blood pressure, pulse rate, respiratory rate, and body temperature), ophthalmological examinations, and 12-lead ECGs.

In addition, high-precision QTcF (Fridericia's method) analysis using 12-lead ECG recording will be conducted from predose (Day 1) to 24 hours postdose (Day 2) in Part 1. A central ECG laboratory will extract ECG recording from the ECG device.

Statistical Methods:

Pharmacokinetics

Part 1:

Pharmacokinetic parameters will be calculated based on the plasma and urine concentrations of S-648414 by using the non-compartmental analysis. Dose proportionality, dose dependency, and food effect for S-648414 will be examined by using a power model or an analysis of variance (ANOVA). Further details on PK analyses will be included in a separate document.

Part 2:

Pharmacokinetic parameters will be calculated based on the plasma and urine concentrations of S-648414 by using the non-compartmental analysis. Dose proportionality, dose dependency, accumulation ratio, effect of multiple doses, and effect of S-648414 on midazolam PK will be examined by using an ANOVA. Further details on PK analyses will be included in a separate document.

Part 3:

The effects of S-648414 on the PK of dolutegravir and the effects of dolutegravir on the PK of S-648414 will be assessed by an ANOVA model. Further details on PK analyses will be included in a separate document.

Safety

The number of treatment-emergent AEs and the number of study participants who have experienced any treatment-emergent AEs will be summarized by treatment group. The summarization for treatment-related AEs will be performed in a similar manner. Physical examinations, ophthalmological examinations, clinical laboratory tests, vital signs, and 12-lead ECGs will also be summarized.

Cardiodynamic Electrocardiogram Evaluation

The primary analysis will be based on concentration-QTc modeling of the relationship between the concentrations of S-648414 and change-from-baseline QTcF (ΔQTcF) with the intent to exclude an effect > 10 msec at clinically relevant S-648414 plasma concentrations. In addition, the effect of S-648414 on the placebo-corrected ΔQTcF, ΔHR (heart rate), ΔPR, and ΔQRS (ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS) will be evaluated at each postdosing time point ("by-time point" analysis) using the Intersection Union Test. An analysis of categorical outliers will be performed for changes in HR, PR, QRS, QTcF, T-wave morphology and U-wave presence.

Table 1-5 Sequence of Steps

Group	Sequence										
Part 1 – Single dosing after overnight fast of ≥ 10 hours											
Group A-1[a] (10 mg)	Step 1-1										
Group A-2[a] (10 mg)		Step 1-2									
Group B (30 mg)			Step 2								
Group C[b] (100 mg)				Step 3-1 Fasted	Step 3-2 Fed (30 minutes after the initiation of breakfast)						
Group D (250 mg)					Step 4						
Group E (500 mg)						Step 5					
Group F (1000 mg)							Step 6				
Part 2– Multiple dosing administered 30 minutes after the initiation of breakfast											
Group G (50 mg)								Step 7[c] MDZ			
Group H (30 mg)									Step 8[c] MDZ		
Part 3 – Multiple dosing administered 30 minutes after the initiation of breakfast											
Group I (100 mg)										Step 9[d]	
Group J (200 mg)											Step 10[d]

MDZ = midazolam

a The initial dose group (Group A) is divided into Group A-1 including 2 study participants (1 S-648414 and 1 placebo) and Group A-2 including 6 study participants (5 S-648414 and 1 placebo). Study participants in Group A-2 will receive study intervention (S-648414 or placebo) in a sentinel dosing sequence, ie, only after confirmation that there are no clinical concerns in Group A-1 within 24 hours postdose.

b Group C-1 is the same as Step 3-1, and will be followed sequentially by Group C-2 (Step 3-2). For Group C, study participants will receive the first dose of study intervention (100 mg) in a fasted state (Group C-1), followed by a ≥ 14 days washout period and then the second dose of study intervention (100 mg) in a fed state (Group C-2).

c Steps 7 and 8 will include administration of MDZ on Day -2 and Day 14 and multiple administrations of S-648414 or matching placebo.

d Steps 9 and 10 will include administration of dolutegravir 50 mg once daily for 7 days, followed by a washout period of at least 7 days, followed by administration of S-648414 for 7 days, followed by coadministration of S-648414 and dolutegravir 50 mg once daily for 7 days.

1.2 Schema

See Schedule of Activities for Part 1 (Figure 1-2), Part 2 (Figure 1-3), and Part 3 (Figure 1-4 and Figure 1-5).

The study schematic in Part 3 is summarized below in Figure 1-1.

Figure 1-1 Study Schematic – Part 3

Screening Period	**Period 1 (Confinement in CRU)**	**No Treatment**	**Period 2 (Confinement in CRU)**	**Period 3 (Confinement in CRU)**	**Follow-up Period**
→	Dolutegravir QD →	No treatment →	S-648414 QD →	S-648414 QD + dolutegravir QD →	→
4 weeks (Day -28 to Day -2)	9 days (Day -1[a] to Day 8[b])	At least 5 days (Day 9 to Day 13)[c]	8 days (Day 14[d] to Day 21)	8 days (Day 22 to Day 29[b])	7 days after discharge of Period 3 (Day 36) ± 2 days

CRU = clinical research unit; QD = once daily

a Admission day is on Day -1; first day of dolutegravir is on Day 1.

b Discharge from CRU (if applicable); no study intervention administered on day of discharge.

c Study participant location (ie CRU or outpatient) will be at the discretion of the investigator.

d Confinement in CRU starts on Day 14 and first day of S-648414 is Day 15.

1.3 Schedule of Activities

Figure 1-2 Schedule of Activities – Part 1

Procedure	Screening Day	Admission Day	Confinement in CRU (Day)[a]				Discharge from CRU (Day)	Follow-up or Early Termination Visit (Day)
	−28 to −2	−1	1	2	3	4	5	10 ± 2
Administrative assessments								
Informed consent	X							
Inclusion/exclusion criteria	X	X	X					
Demographics	X							
Medical history including prior medications[b]	X	X						
Clinical assessments								
Height, weight, BMI[c]	X	X					X	X
Vital signs[d]	X	X	X	X	X	X	X	X
Physical examination	X	X					X	X
Symptom-focused physical examination[e]			X	X	X	X		
ECG for safety assessment[f]	X	X	X	X			X	X
Cardiodynamic evaluation[g]			X	X				
Ophthalmological examinations: fundoscopy, tonometry (intraocular pressure), and vision acuity test[h]	X	x					X	X
Adverse events	←							→
Gastrointestinal adverse events[i]	←							→
Liver events collection	←							→

Procedure	Screening Day −28 to −2	Admission Day −1	Confinement in CRU (Day)[a] 1	 2	 3	 4	Discharge from CRU (Day) 5	Follow-up or Early Termination Visit (Day) 10 ± 2
Concomitant medication monitoring	←	———	———	———	———	———	———	→
Special situations (abuse, misuse, overdose, or medication error of the study intervention)		←	———	———	———	———	———	→
Laboratory assessments								
Drug and alcohol screen	X	X						
Serological tests[j]	X							
Pregnancy test – urine[k]	X	X						
FSH test[l]	X							
Laboratory tests[m]	X	X		X	X		X	X
PK blood samples[n]			X	X	X	X	X	
PGx sample collection[o]		X						
PK urine samples[p]		X	X	X	X	X	X	
Metabolite profiling[q]			X	X	X	X	X	
Study intervention (S-648414 or placebo) procedures								
Randomization		X						
Drug administration[r] **(after 10-hour fast)**			X					
Study-specific assessments								
Group C, second dose (C-2 - fed) **- dosed 30 minutes after the initiation of high fat breakfast**[s]			X					

BMI = body mass index; CRU = clinical research unit; ECG = electrocardiogram; FSH = follicle-stimulating hormone; HIV = human immunodeficiency virus; PGx = pharmacogenomics; PK = pharmacokinetic

a Study participants will be admitted to the CRU the day prior to the administration of study intervention (S-648414 or placebo) in the morning on Day -1 and discharged on Day 5 after the completion of all study procedures.

b Medical history includes a review of prior therapies (30 days prior to Screening visit).

c Height will be measured only at Screening.

d Vital signs include oral body temperature, pulse rate (1 measurement), respiratory rate, and blood pressure (3 consecutive readings) collected at Screening, Admission Day (Day -1), Day 1: predose (0 hours), 1.5, 3, 5, 8, and 12 hours postdose, Day 2: 24 hours postdose, Day 3: 48 hours postdose, Day 4: 72 hours postdose, Day 5: 96 hours postdose, and Follow-up (Days 8 to 12) or Early Termination visit. Allowable time frame for collection is ± 20 minutes (2 to 0 hours for the predose collection).

e Symptom-focused physical examinations include updates only.

f Electrocardiograms for safety assessment at Screening, Admission Day (Day -1), Day 1: predose (0 hours), 1, 3, 5, and 12 hours postdose, Day 2: 24 hours postdose, Day 5: 96 hours postdose, and Follow-up (Days 8 to 12) or Early Termination visit. Allowable time frame for collection is ± 8 minutes (2 to 0 hours for the predose collection).

g Cardiodynamic evaluations include High-precision 12-lead ECG tracings will be extracted from Holter recordings during the following time points: Day 1: Predose at 3 time points (-45, -30 and -15 minutes), and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 hours postdose. For Group C, ECG will not be collected in Step 3-2. Electrocardiograms will be recorded after vital signs and prior to blood draws. Study participants will need to rest in the supine position for 10 minutes before and during the vital signs, ECGs, and blood draws and the rest must be documented in source notes. Allowable time frame for collection is ± 8 minutes (2 to 0 hours for the predose collection).

h Ophthalmological assessments will be carried out by an ophthalmologist at the specified time points.

i Additional data collection required for gastrointestinal adverse events: nausea, vomiting, diarrhea, and abdominal pain.

j Serological tests include testing for positive syphilis, positive hepatitis B surface antigen, positive hepatitis C virus antibody, and positive HIV antigen/antibody.

k Urine pregnancy test is only for females who are not diagnosed as postmenopausal.

l Blood sample for serum FSH levels must be obtained to confirm female study participant's postmenopausal status if there is no available documentation confirming postmenopausal status.

m Laboratory tests include hematology, blood chemistry, and urinalysis.

n Pharmacokinetic blood samples collected at predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 12 hours postdose (Day 1), 24 hours postdose (Day 2), 48 hours postdose (Day 3), 72 hours postdose (Day 4), and 96 hours postdose (Day 5). The PK blood sampling schedule may be changed according to the PK results in the preceding group(s). Allowable time frame for collection is ± 5 minutes, with the exception of the samples collected ≥ 24 hours postdose which is ± 30 minutes.

o For study participants who agree to participate in the PGx research, these samples should be obtained on Admission Day (Day -1).

p Pharmacokinetic urine samples collected at predose (-12 to 0 hours), 0 to 24 hours, 24 to 48 hours, 48 to 72 hours, and 72 to 96 hours postdose. The time intervals for urine sampling may be changed according to the PK results in the preceding group(s).

q Metabolite profiling collected at predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 12 hours postdose (Day 1), 24 hours postdose (Day 2), 48 hours postdose (Day 3), 72 hours postdose (Day 4), and 96 hours postdose (Day 5) for plasma samples. Metabolite profiling collected at predose (-12 to 0 hours), 0 to 24 hours, 24 to 48 hours, 48 to 72 hours, and 72 to 96 hours postdose for urine samples.

r Study intervention (S-648414 or placebo) will be administered after an overnight fast of at least 10 hours. For group C, the second dose (Step 3-2) will be administered 30 minutes after the initiation of breakfast (high-fat meal).

s For Group C, study participants will receive the first dose of study intervention (100 mg) in a fasted state (Group C-1), followed by a ≥ 14 days washout period and then the second dose of study intervention (100 mg) in a fed state (Group C-2).

For the group of study participants who will be assessed for the effect of food (Group C), Step 3-1 and Step 3-2 study procedures will be started on Day -1.

Unless otherwise stated, predose procedures will be completed within approximately 1 hour of dosing and approximately ± 10 minutes for all postdose procedures.

Figure 1-3 Schedule of Activities – Part 2, Groups G and H

	Screening Day	Admission Day	Confinement in CRU (Day)[a]																			Discharge from CRU (Day)	Follow-up or Early Termination Visit (Day)
Procedure	**−28 to −4**	**−3**	**-2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**25 ± 2**
Administrative assessments																								
Informed consent	X																							
Inclusion/exclusion criteria	X	X	X																					
Demographics	X																							
Medical history including prior medications[b]	X	X																						
Clinical assessments																								
Height, weight, BMI[c]	X	X																					X	X
Vital signs[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X																					X	X
Symptom-focused physical examination[e]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
ECG for safety assessment[f]	X	X	X		X	X			X			X			X			X					X	X
Ophthalmological examinations: fundoscopy, tonometry (intraocular pressure), and vision acuity test[g]	X	X																X					X	X
Adverse events	←																					→		
Gastrointestinal adverse events[h]	←																					→		

Procedure	Screening Day −28 to −4	Admission Day −3	Confinement in CRU (Day)[a] -2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Discharge from CRU (Day) 19	Follow-up or Early Termination Visit (Day) 25 ± 2
Liver events collection	←	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	→
Concomitant medication monitoring	←	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	→
Special situations (abuse, misuse, overdose, or medication error of the study intervention)			←	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	→
Laboratory assessments																								
Drug and alcohol screen	X	X																						
Serological tests[i]	X																							
Pregnancy test –urine[j]	X	X																						
FSH test[k]	X																							
Laboratory tests[l]	X	X		X		X			X			X			X			X					X	X
PK blood samples[m]			X	X	X	X			X		X			X		X	X	X	X	X	X	X		
PGx sample collection[n]		X																						
PK urine samples[o]																		X						
Biomarker sample[p]			X															X						
Study intervention procedures																								
Randomization		X																						
Drug administration (midazolam)			X															X						
Drug administration (S-648414 or placebo)[q]					X	X	X	X	X	X	X	X	X	X	X	X	X	X						

BMI = body mass index; CRU = clinical research unit; ECG = electrocardiogram; FSH = follicle-stimulating hormone; HIV = human immunodeficiency virus; PGx = pharmacogenomics; PK = pharmacokinetic

a Study participants will be admitted to the CRU the day prior to the administration of midazolam and discharged on Day 19 after the completion of all study procedures.

b Medical history includes a review of prior therapies (30 days prior to Screening visit).

c Height will be measured only at Screening.

d Vital signs include oral body temperature, pulse rate (1 measurement), respiratory rate, and blood pressure (3 consecutive readings) collected at Screening, Admission Day (Day -3), Day -2: predose (0 hours) and 1.5 hours postdose, Day -1: Morning, Day 1: predose (0 hours), 0.5, 1.5, 3, 6, 8, and 12 hours postdose, Day 2 to Day 13: predose (0 hours), Day 14: predose (0 hours), 1.5, 6, and 12 hours postdose, Day 15 to 18: Morning, Day 19: 120 hours postdose (at discharge), and Follow-up (Day 23 to 27) or Early Termination visit. Allowable time frame for collection is ± 20 minutes (2 to 0 hours for the predose collection).

e Symptom-focused physical examinations include updates only.

f Electrocardiograms for safety assessment at Screening, Admission Day (Day -3), Day -2: predose (0 hours), 1.5, 6, and 12 hours postdose, Day 1: predose (0 hours), 1.5, 6, and 12 hours postdose, Days 2, 5, 8, 11, and 14: predose (0 hours) and 1.5 hours postdose, Day 19:120 hours postdose (at discharge), Follow-up (Day 23 to 27) or Early Termination visit. Allowable time frame for collection is ± 8 minutes (2 to 0 hours for the predose collection).

g Ophthalmological assessments will be carried out by an ophthalmologist at the specified time points.

h Additional data collection required for gastrointestinal adverse events: nausea, vomiting, diarrhea, and abdominal pain.

i Serological tests include testing for positive syphilis, positive hepatitis B surface antigen, positive hepatitis C virus antibody, and positive HIV antigen/antibody.

j Urine pregnancy test is only for females who are not diagnosed as postmenopausal.

k Blood sample for serum FSH levels must be obtained to confirm female study participant's postmenopausal status if there is no available documentation confirming postmenopausal status.

l Laboratory tests include hematology, blood chemistry, and urinalysis.

m Pharmacokinetic blood samples collected at:

Day -2 (midazolam): predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hours postdose.

Day 1 (S-648414): predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hours postdose.

Days 5, 7, 10, 12, and 13 (S-648414): predose (0 hours).

Day 14 (midazolam): predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hours postdose.

Day 14 (S-648414): predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 24, 48, 72, and 96 hours postdose.

Allowable time frame for collection is ± 5 minutes and ≥ 24 hours after dosing is ± 30 minutes.

n For study participants who agree to participate in the PGx research only, these samples should be obtained on Admission Day (Day -3).

o Pharmacokinetic urine sample collected on Day 14 only (0 to 24 hours postdose).

p Biomarker sample collected on Day -2 and Day 14 (4 and 24 hours postdose). Allowable time frame for collection is ± 10 minutes, with the exception of the samples collected ≥ 24 hours after dosing which is ± 30 minutes.

q The study intervention (S-648414 or placebo) will be administered 30 minutes after the initiation of breakfast.

Unless otherwise stated, predose procedures will be completed within approximately 1 hour of dosing and approximately ± 10 minutes for all postdose procedures.

Figure 1-4 Schedule of Activities – Part 3, Period 1, Groups I and J

	Screening Day	Admission Day	Confinement in CRU (Day)[a]							Discharge from CRU (Day)[b]	Outpatient[b] (Day)
		Period 1									
Procedure	−28 to −2	−1	1	2	3	4	5	6	7	8	At least 5 days (Day 9 to Day 13)
Administrative assessments											
Informed consent	X										
Inclusion/exclusion criteria	X	X									
Demographics	X										
Medical history including prior medications[c]	X	X									
Clinical assessments											
Height, weight, BMI[d]	X	X									
Vital signs[e]	X	X	X							X	
Physical examination	X	X								X	
Symptom-focused physical examination[f]			X	X	X	X	X	X	X		
ECG for safety assessment[g]	X	X	X							X	
Ophthalmological examinations: fundoscopy, tonometry (intraocular pressure), and vision acuity test[h]		X									
Adverse events	←										→
Gastrointestinal adverse events[i]	←										→
Liver events collection	←										→
Concomitant medication monitoring	←										→

Procedure	Screening Day	Admission Day	Confinement in CRU (Day)[a]							Discharge from CRU (Day)[b]	Outpatient[b] (Day)
		Period 1									
	−28 to −2	−1	1	2	3	4	5	6	7	8	At least 5 days (Day 9 to Day 13)
Special situations (abuse, misuse, overdose, or medication error of the study intervention)	←										→
Laboratory assessments											
Drug, alcohol and cotinine screen	X	X									
Serological tests[j]	X										
Pregnancy test - urine[k]	X	X									
FSH test[l]	X										
Laboratory tests[m]	X	X								X	
PK blood samples[n]			X			X	X	X	X	X	
PGx sample collection[o]		X									
Dolutegravir procedures											
Drug administration[p]			X	X	X	X	X	X	X		

BMI = body mass index; CRU = clinical research unit; ECG = electrocardiogram; FSH = follicle-stimulating hormone; HIV = human immunodeficiency virus; PGx = pharmacogenomics; PK = pharmacokinetic

a Study participants will be admitted to the CRU the day prior to the administration of dolutegravir in the morning on Day −1 and discharged on Day 8 (if applicable).

b Study participant location from Days 9 to 13 will be at the discretion of the investigator.

c Medical history includes a review of prior therapies (30 days prior to Screening visit).

d Height will be measured only at the Screening.

e Vital signs include body temperature, pulse rate (1 measurement), respiratory rate, and blood pressure (3 consecutive readings) collected at Screening, Admission Day (Day −1), Day 1: predose, 1.5 hours postdose, and Day 8: 24 hours postdose, and Early Termination. Allowable time frame for collection is ± 20 minutes (2 to 0 hours for the predose collection).

Procedure	Screening Day	Admission Day	Confinement in CRU (Day)[a]							Discharge from CRU (Day)[b]	Outpatient[b] (Day)
		Period 1									
	−28 to −2	−1	1	2	3	4	5	6	7	8	At least 5 days (Day 9 to Day 13)

f Symptom-focused physical examinations should be conducted for new or worsening adverse events/symptoms.

g Electrocardiograms for safety assessment at Screening, Admission Day (Day −1), Day 1: predose, 1.5 hours postdose, Day 8: 24 hours postdose, and Early Termination. Allowable time frame for collection is ± 8 minutes (2 to 0 hours for the predose collection).

h Ophthalmological assessments will be carried out by an ophthalmologist at the specified time points.

i Additional data collection required for gastrointestinal adverse events: nausea, vomiting, diarrhea, and abdominal pain.

j Serological tests include testing for positive syphilis, positive hepatitis B surface antigen, positive hepatitis C virus antibody, and positive HIV antigen/antibody.

k Urine pregnancy test is only for females who are not diagnosed as postmenopausal.

l Blood sample for serum FSH levels may be obtained to confirm female study participant's postmenopausal status if there is no available documentation confirming postmenopausal status.

m Laboratory tests include hematology, blood chemistry, and urinalysis.

n Pharmacokinetic blood samples collected at: Dolutegravir: Day 1: Pre-dose (0 hours), Day 4 to 6: Pre-dose (0 hours), Day 7: Predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hours postdose (Day 8). Allowable time frame for collection is ± 5 minutes and ≥ 24 hours after dosing is ± 30 minutes.

o For study participants who agree to participate in the PGx research only, these samples should be obtained at Admission Day (Day −1).

p Dolutegravir will be administered 30 minutes after the initiation of breakfast.

Unless otherwise stated, predose procedures will be completed within approximately 1 hour of dosing and approximately ± 10 minutes for all postdose procedures.

Figure 1-5 Schedule of Activities – Part 3, Periods 2 and 3, Groups I and J

	Admission Day	Confinement in CRU (Day)[a]														Discharge from CRU (Day)	Follow-up or Early Termination Visit (Day)[b]
		Period 2							Period 3								
Procedure	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	7 days after discharge of Period 3 (Day 36) ± 2 days
Administrative assessments																	
Informed consent																	
Inclusion/exclusion criteria	X																
Demographics																	
Medical history and prior medications[c]	X																
Clinical assessments																	
Height, weight, BMI[d]	X															X	
Vital signs[e]	X	X						X	X						X	X	X
Physical examination	X															X	X
Symptom-focused physical examination[f]		X	X	X	X	X	X	X	X	X	X	X	X	X	X		
ECG for safety assessment[g]	X	X	X					X	X						X	X	X
Ophthalmological examinations: fundoscopy, tonometry (intraocular pressure), and vision acuity test[h]																X	
Adverse events	←																→
Gastrointestinal adverse events[i]	←																→

Procedure	Admission Day	Confinement in CRU (Day)[a]														Discharge from CRU (Day)	Follow-up or Early Termination Visit (Day)[b]
		Period 2							Period 3								
	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	7 days after discharge of Period 3 (Day 36) ± 2 days
Liver events collection	←	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	→
Concomitant medication monitoring	←	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	→
Special situations (abuse, misuse, overdose, or medication error of the study intervention)	←	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	→
Laboratory assessments																	
Drug and alcohol screen	X																
Pregnancy test – urine[j]	X																
Laboratory tests[k]	X							X							X	X	X
PK blood samples[l]		X			X	X	X	X	X			X	X	X	X	X	
PGx sample collection																	
Study intervention procedures																	
Drug administration[m]		X	X	X	X	X	X	X	X	X	X	X	X	X	X		

BMI = body mass index; CRU = clinical research unit; ECG = electrocardiogram; FSH = follicle-stimulating hormone; HIV = human immunodeficiency virus; PGx = pharmacogenomics; PK = pharmacokinetic

a Study participants will be admitted to the CRU the day prior to the administration of study intervention (S-648414) in the morning on Day 14 and discharged on Day 29.

b For study participants who are withdrawn from the study, the Early Termination assessment should be performed within 7 to 11 days after the last administration of study intervention, if possible.

c Medical history includes a review of prior therapies (30 days prior to Screening visit).

d Height will be measured only at Screening.

e Vital signs include body temperature, pulse rate (1 measurement), respiratory rate, and blood pressure (3 consecutive readings) collected at Admission Day (Day 14), Days 15, 21, 22, and 28: predose, Day 29: 24 hours postdose, and Follow-up visit or Early Termination. Allowable time frame for collection is ± 20 minutes (2 to 0 hours for the predose collection).

f Symptom-focused physical examinations should be conducted for new or worsening adverse events/symptoms.

g Electrocardiograms for safety assessment at Admission Day (Day 14), Days 15, 21, 22, and 28: predose, 1.5 hours postdose, Day 16: 24 hours postdose, Day 29 and Follow-up visit or Early Termination. Allowable time frame for collection is ± 8 minutes (2 to 0 hours for the predose collection).

h Ophthalmological assessments will be carried out by an ophthalmologist at the specified time points.

i Additional data collection required for gastrointestinal adverse events: nausea, vomiting, diarrhea, and abdominal pain.

j Urine pregnancy test is only for females who are not diagnosed as postmenopausal.

k Laboratory tests include hematology, blood chemistry, and urinalysis.

l Pharmacokinetic blood samples collected.

In Period 2, S-648414: Day 15: Pre-dose (0 hours), Day 18 to 20: Pre-dose (0 hours), Day 21: Predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 12 hours postdose.

In Period 3, S-648414 and dolutegravir: Day 22: Pre-dose (24 hours after Day 21 dose), Day 25 to 27: Pre-dose (0 hours), Day 28: Predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hours postdose (Day 29).

Allowable time frame for collection is ± 5 minutes and ≥ 24 hours after dosing is ± 30 minutes.

m Study intervention (S-648414) will be administered during Period 2 and study intervention (S-648414 and dolutegravir) will be administered during Period 3 to study participants in a fed state (ie, 30 minutes after initiation of breakfast).

Unless otherwise stated, predose procedures will be completed within approximately 1 hour of dosing and approximately ± 10 minutes for all postdose procedures.

2. INTRODUCTION

2.1 Study Rationale

S-648414 is a novel antiretroviral agent that is being developed as a treatment for human immunodeficiency virus (HIV-1) infection in combination with other antiretroviral agents to fully suppress HIV-1 viremia. Based on a full pre-clinical development program of S-648414 this study is expected to be conducted safely and the minimum and maximum doses tested have been determined based on in-vitro efficacy estimations and animal Good Laboratory Practice (GLP)-compliant toxicology studies carried out to GLP standards. These Phase 1 assessments will describe the safety, tolerability, and pharmacokinetics (PK) of S-648414 following first-in-human dosing, both single ascending and multiple ascending dosing (SAD/MAD), food effect, effect on electrocardiogram (ECG) parameters, and effect on the PK of midazolam, a cytochrome P450 3A (CYP3A) probe, as well as the effect on PK of dolutegravir and the effect of dolutegravir on PK of S-648414. The study will permit evaluation of dosing and safety in the clinical setting to inform and facilitate studies in individuals living with HIV-1 infection.

2.2 Background

S-648414 inhibits HIV-1 replication via binding to a conserved allosteric pocket of the HIV-1 integrase (IN) enzyme known as an IN-lens epithelium-derived growth factor (LEDGF) binding pocket [1] and may cause abnormal IN multimerization resulting in inhibiting the viral particle maturation which is necessary for replication of HIV-1. This mechanism of action differs from all approved HIV treatments.

HIV-1 infection, although incurable, can be effectively treated with combination antiretroviral therapy, usually consisting of 3 agents [2]. Combination antiretroviral therapy, when instituted early and with high levels of patient adherence, controls plasma viremia and leads to immune recovery with resulting reductions in morbidity and mortality [3]. A patient diagnosed early and treated effectively may have a normal life expectancy [4] and this treatment is also effective at preventing HIV transmission [5]. The efficacy of treatment may be compromised by reduced patient adherence, or the presence of preexisting antiretroviral resistance, which may be transmitted resistance [6] or reflect previous nonsuppressive/suboptimal therapy [7]. Some patients living with HIV were serially exposed to suboptimal therapy in the form of monotherapy or dual therapy as treatment paradigms evolved and new therapies became available. For these patients, efficacy of combination therapy instituted later was compromised by lack of sensitivity to some components of the regimen [8]. Reduced patient adherence can be driven by poor tolerability or toxicity problems with existing treatments [9]. Therefore, there is a need for new classes of antiretrovirals that overcome preexisting resistance and are better tolerated, with reduced pill burden and dosing frequency.

Based on the nonclinical data obtained to date, S-648414 has shown comparable or better inhibitory effects on viral replication than those of other commercialized or investigational anti-HIV-1 drugs and no significant effect on various receptors, ion channels, transporters, or enzymes. S-648414 has also shown a largely good PK profile in

rats and monkeys. Radioactivity was detected in melanin-containing tissues (the choroid, ciliary body, and iris) at the final sampling time (840 hours postdose) in a pigmented rat quantitative whole-body autoradiography (QWBA) study; however, there was no toxic finding on eyes in 4-week toxicity studies in rats and monkeys. S-648414 was shown to be a substrate of P-glycoprotein and in vitro studies demonstrated an induction potential of cytochrome P450 (CYP) 1A2, CYP2B6, and CYP3A4.

No safety concerns have arisen in the core battery of safety pharmacology studies. The potential of genotoxicity was considered low. In 2-week toxicity studies in rats treated with up to 300 mg/kg/day and monkeys treated with up to 1000 mg/kg/day, the target organs were the kidney, gastrointestinal (GI) tracts in rats and monkeys, and the liver in monkeys. In addition, slight prolongation of corrected QT interval (QTc) was also noted in monkeys at 1000 mg/kg/day. In 4-week toxicity studies in rats and monkeys treated up to 200 mg/kg/day, the adverse findings included infiltration of inflammatory cells and degeneration in the mucosa of the glandular stomach in rats and vomiting and increased plasma hepatobiliary parameters accompanied with hepatocellular vacuolation in monkeys. All of the findings showed reversibility after 4-week drug withdrawal. The no observed adverse levels (NOAEL) in rats and monkeys were estimated to be 30 mg/kg/day (4.8 mg/kg as human equivalent dose [HED]) and 60 mg/kg/day (19.2 mg/kg as HED), respectively. From these data, it is considered that S-648414 has an acceptable safety profile for proceeding into clinical studies.

This first-in-human Phase 1 study will assess the safety, tolerability, and PK of S-648414 following both single ascending and multiple ascending dosing (SAD/MAD); it will also assess for a food effect, effect on ECG parameters, and effect on the PK of midazolam, a CYP3A probe. S-648414 will be administered as single oral doses up to 1000 mg and as repeated oral doses up to 50 mg once daily (QD) for 14 days. The study will also assess the safety and tolerability of multiple doses of S-648414 (100 mg and 200 mg) and the effect of these doses of S-648414 on the PK of dolutegravir and the effect of dolutegravir (50 mg) on the PK of S-648414.

2.3 Benefit/Risk Assessment

S-648414 is an investigational agent and this study represents the first human exposure, Since S-648414 is intended as an antiretroviral agent for the treatment of HIV, there is no benefit for study participants.

More detailed information about the potential risks and reasonably expected AEs of S-648414 may be found in the Investigator’s Brochure.

3. OBJECTIVES AND ENDPOINTS

Table 3-1 Objectives and Endpoints

Objectives	Endpoints
Primary	
Part 1: • To evaluate the safety and tolerability of S-648414 after administration of a single oral dose of S-648414 in healthy adult study participants **Part 2:** • To evaluate the safety and tolerability of S-648414 after administration of multiple oral doses of S-648414 in healthy adult study participants **Part 3:** • To evaluate the safety and tolerability of S-648414 after administration of multiple oral doses of S-648414 in healthy adult study participants • To evaluate the effect of S-648414 on the PK of dolutegravir in healthy adult study participants • To evaluate the effect of dolutegravir on the PK of S-648414 in healthy adult study participants	**Part 1:** • The number and percentage of clinical and laboratory adverse events in study participants exposed to single dose of S-648414 **Part 2:** • The number and percentage of clinical and laboratory adverse events in study participants exposed to multiple doses of S-648414 **Part 3:** • The number and percentage of clinical and laboratory adverse events in study participants exposed to multiple doses of S-648414 • S-648414 and dolutegravir: C_{max}, T_{max}, C_{τ}, AUC, and CL/F
Secondary	
Part 1: • To assess the PK of S-648414 after administration of a single oral dose of S-648414 in healthy adult study participants • To assess the effect of a high-fat meal on the PK of S-648414 after administration of a single oral dose of S-648414 in healthy adult study participants • To assess the effect of S-648414 on ECG parameters in healthy adult study participants **Part 2:** • To assess the PK of S-648414 after administration of multiple oral doses of S-648414 in healthy adult study participants • To evaluate the potential of S-648414 for inhibition or induction of the CYP3A enzymes using MDZ as a probe substrate after administration of multiple oral doses of S-648414 in healthy adult study participants **Part 3:** • To evaluate the safety and tolerability after coadministration of dolutegravir with S-648414 in healthy adult study participants	**Parts 1 and 2:** • S-648414: C_{max}, T_{max}, AUC, $t_{1/2,z}$, λ_z, MRT, CL/F, V_z/F, CL_R, and Feu following single oral dosing, multiple dosing and in fasted and fed state • Change from-baseline in HR, QTcF, PR and QRS (ΔHR, ΔQTcF, ΔPR and ΔQRS) • Placebo-corrected ΔHR, ΔQTcF, ΔPR and ΔQRS (ΔΔHR, ΔΔQTcF, ΔΔPR and ΔΔQRS) • Categorical outliers for HR, QTcF, PR, QRS • Frequency of treatment-emergent changes for T-wave morphology and U-wave presence **Part 2 only:** • MDZ: C_{max}, T_{max}, AUC, $t_{1/2,z}$, λ_z, and MRT **Part 3 only:** • The number and percentage of clinical and laboratory adverse events in study participants exposed to multiple doses of S-648414 and dolutegravir

AUC = area under the concentration-time curve; C_τ = plasma concentration at the end of the dosing interval τ (24 hours); CL/F = apparent total clearance; CL_R = renal clearance; C_{max} = maximum plasma concentration; CYP3A = cytochrome P450 3A; ECG = electrocardiogram; Feu = fraction of dose excreted in urine; HR = heart rate; MDZ = midazolam; MRT = mean residence time; PK = pharmacokinetics; PR = measure between beginning of P wave until beginning of QRS complex; QRS = combination of the Q, R, and S waves; QTcF = Fridericia's corrected QT; T_{max} = time to maximum plasma concentration; $t_{1/2,z}$ = terminal elimination half-life; λ_z = terminal elimination rate constant; Vz/F = apparent volume of distribution in the terminal elimination phase; Δ = change from baseline; ΔΔ = placebo-corrected change from baseline

4. STUDY DESIGN

4.1 Overall Design

The study is a multicenter, randomized, double-blind (Parts 1 and 2) and open-label (Part 3), placebo-controlled, sequential group study in healthy adult study participants.

The study consists of Part 1 (single ascending dose, food effect, and effects on ECG parameters including concentration-QTc analysis) and Part 2 (multiple dose and drug-drug interaction with the CYP3A substrate), and Part 3 (the effect of S-648414 on the PK of dolutegravir and the effect of dolutegravir on the PK of S-648414). The sequence of steps in Parts 1, 2, and 3 are summarized in Table 6-3.

Part 1:

The study consists of a Screening period (Days −28 to −2), admission to the clinical research unit (CRU, Day −1), single administrations of S-648414 or matching placebo (Day 1), discharge from the CRU (Day 5), and a Follow-up visit (Day 10 ± 2) (see Figure 1-2).

In Groups A, B, D, E, and F, eligible healthy adult study participants will be assigned to 1 of 5 ascending dose groups (10 to 1000 mg) and receive a single oral dose of S -648414 or placebo in a fasted state with 6 study participants per dose group receiving active drug and 2 study participants per dose group receiving placebo. Administration initiates from the lowest dose group, and the next group does not initiate until the Follow-up period has been completed for the previous group except for A-2 (see Table 4-1).

In Group C, 8 study participants will be assigned to S-648414, and 2 study participants will be assigned to placebo. Study participants assigned to the 100-mg group will receive a single oral dose of S-648414 in a fasted state (Group C-1), followed by a single dose of S-648414 in a fed state (high-fat meal) (Group C-2). Each episode of dosing will be separated by a washout period of at least 14 days.

Table 4-1 Part 1 Dose Groups

Group Label	S-648414 Dose	Number of Active Study Participants	Number of Placebo Study Participants
Group A (Group A-1 and A-2)[a]	10 mg	6	2
Group B	30 mg	6	2
Group C (Group C-1 and C-2)	100 mg	8	2
Group D	250 mg	6	2
Group E	500 mg	6	2
Group F	1000 mg	6	2

a Because this is a first-in-human administration, the initial dose group (Group A) is divided into Group A-1 including 2 study participants (1 S-648414 and 1 placebo) and Group A-2 including 6 study participants (5 S-648414 and 1 placebo). Study participants in Group A-2 will receive study intervention (S-648414 or placebo) in a sentinel dosing sequence, ie, only after confirmation that there are no clinical concerns in Group A-1 within 24 hours postdose.

The doses may be changed depending on the results of the safety, tolerability, and PK in the preceding group(s), if necessary. The highest dose will not exceed 1000 mg.

The investigator **and** the sponsor will evaluate the results of the safety and tolerability in the preceding group(s) and decide whether to proceed to the next dose group.

The next dose group will not be conducted if any of the following criteria are met:

- Any study participant experiences a treatment-related SAE (ie, treatment-emergent SAE considered related to the study intervention [S-648414 or placebo] by the investigator and sponsor)
- ≥ 50% of study participants experience grade 2 to 4 treatment-related AEs
- ≥ 25% of study participants experience grade 3 or 4 treatment-related AEs

See Section 10.3 (Appendix 3) for definitions of AEs, SAEs, assessment of intensity (including HIV grading system), assessment of causality, and procedures for recording, evaluating, follow-up, and reporting.

Part 2:

Eligible healthy adult study participants are assigned to 1 of 2 dose groups (50 mg in Group G or 30 mg in Group H) and receive multiple oral doses of S-648414 tablet or placebo in a fed state with 8 study participants receiving active drug and 2 study participants receiving placebo per group (see Table 4-2). Administration of the next dose group (Group H) will not initiate until the Follow-up period has been completed for the previous group (Group G).

In Groups G and H, the study consists of a Screening period (Days −28 to −4), admission to the CRU (Day −3), administration of midazolam 5 mg (Day −2 and Day 14)

and multiple administrations of S-648414 or matching placebo (Days 1 to 14), discharge from the CRU (Day 19), and a Follow-up visit (Day 25 ± 2) (see Figure 1-3).

All study participants will receive a single oral dose of midazolam 5 mg alone on Day -2 and coadministered with the S-648414/placebo dose on Day 14. Study participants will receive S-648414/placebo QD on Days 1 to 14 (Group G will receive 50 mg and Group H will receive 30 mg).

Table 4-2 Part 2 Dose Groups

Group Label	S-648414 Dose	Number of Active Study Participants	Number of Placebo Study Participants	Length of Treatment (Days)
Group G	50 mg	8	2	14
Group H	30 mg	8	2	14

The doses, mode of administration, frequency (ie, QD or twice daily [BID]), dietary condition (ie, fasted or fed), or length of administration may be changed depending on the results of the safety, tolerability, and PK in the preceding group(s), if necessary.

The investigator **and** the sponsor will evaluate the results of the safety and tolerability in the preceding group(s) and decide whether to proceed to the next dose group.

The next dose group will not be conducted if any of the following criteria are met:

- Any study participant experiences a treatment-related SAE (ie, treatment-emergent SAE considered related to the study intervention [S-648414 or placebo] by the investigator and sponsor)
- ≥ 50% of study participants experience grade 2 to 4 treatment-related AEs
- ≥ 25% of study participants experience grade 3 or 4treatment-related AEs

See Section 10.3 (Appendix 3) for definitions of AEs, SAEs, assessment of intensity (including HIV grading system), assessment of causality, and procedures for recording, evaluating, and follow-up of AEs and SAEs, and reporting SAEs.

Part 3:

Part 3 is an open-label, non-randomized, 1-sequence, 3-period study.

A total of 14 healthy adult study participants will be enrolled in Group I of Part 3; an additional 14 healthy adult study participants will be enrolled in Group J of Part 3.

Part 3 will consist of:

- Screening period (Days −28 to −2)
- Period 1 (admission to CRU on Day -1 with continued confinement until Day 8)

- Mandatory 7-day washout between the last dose of study intervention in Period 1 and the first dose of study intervention in Period 2
  - Day 8 of Period 1 (no dose)
  - Days 9 to 13 (5 days, no dose)

    The 5-day period can be inpatient or outpatient and will be decided at the discretion of the investigator.
  - Day 14 of Period 2 (no dose)
- Period 2 (confinement: Days 14 to 21),
- Period 3 (confinement: Days 22 to 29)
- Follow-up visit (7 days after discharge from the CRU of Period 3 [Day 36] ± 2 days)

Study participants will receive:

- Dolutegravir (50 mg orally QD for 7 days) in the fed state during Period 1
- S-648414 alone (100 mg orally QD for 7 days in Group I or 200 mg orally QD for 7 days in Group J) in the fed state during Study Period 2
- S-648414 (100 mg orally QD for 7 days in Group I or 200 mg orally QD for 7 days in Group J) coadministered with dolutegravir (50 mg orally QD for 7 days) in the fed state during Period 3

See Table 4-3 for the treatment schedule and Figure 1-1 for the study schematic.

Table 4-3 Part 3 Treatment Schedule

Group Label	Number of Study Participants	Period 1[a]	Period 2[a]	Period 3
Group I	14	Dolutegravir 50 mg QD for 7 days	S-648414 100 mg QD for 7 days	S-648414 100 mg + dolutegravir 50 mg QD for 7 days
Group J	14	Dolutegravir 50 mg QD for 7 days	S-648414 200 mg QD for 7 days	S-648414 200 mg + dolutegravir 50 mg QD for 7 days

QD = once daily

a There will be a 7-day washout period between Periods 1 and 2; the 7-day washout period includes the time when the study participant will not receive study intervention on the last day of Period 1 and the first day of Period 2.

The doses, mode of administration, frequency (ie, QD or BID), dietary condition (ie, fasted or fed), or length of administration may be changed by the sponsor depending on the results of the safety, tolerability, and PK in the preceding group(s), if necessary.

The investigator **and** the sponsor will evaluate the results of the safety and tolerability in the preceding group(s) and decide whether to proceed to the next dose group.

The next dose group will not be conducted if any of the following criteria are met:

- Any study participant experiences a treatment-related SAE (ie, treatment-emergent SAE considered related to S-648414 or by the investigator and sponsor)
- ≥ 50% of study participants experience grade 2 to 4 treatment-related AEs
- ≥ 25% of study participants experience grade 3 or 4 treatment-related AEs

See Section 10.3 (Appendix 3) for definitions of AEs, SAEs, assessment of intensity (including HIV grading system), assessment of causality, and procedures for recording, evaluating, follow-up, and reporting.

4.2 Scientific Rationale for Study Design

This Phase 1 study is designed to compare the PK, food effect, and effect on ECG parameters of single and multiple doses of S-648414 with placebo in a double-blind fashion. This is a first-in-human study and will begin with a single escalating dose to ensure safety and tolerability. The study will then progress to a multiple-dosing phase to further define PK parameters. The last phase of the study will include multiple dosing with and without dolutegravir to study effect of S-648414 on the PK of dolutegravir and the effect of dolutegravir on the PK of S-648414.

4.3 Justification for Dose

In 4-week toxicity studies in rats and monkeys, the NOAELs were estimated to be 30 mg/kg/day and 60 mg/kg/day and the HED was calculated as 4.8 mg/kg/day and 19.2 mg/kg/day, respectively. Therefore, rats were used as the most sensitive species.

The HED in rats was divided by a safety factor of 10 to calculate the maximum recommended starting dose (MRSD) as 0.48 mg/kg/day, which corresponds to 28.8 mg/day assuming a body weight of approximately 60 kg in a healthy subject.

The SAD segment of the study will evaluate S-648414 to investigate safety and PK profiles in healthy study participants. After review of the MRSD from the safety evaluation, 10 mg is proposed for the starting dose of S-648414.

In this study, the dose will be increased up to 1000 mg as a single dose. A dose of 1000 mg/human is near the NOAEL in the 4-week toxicity study in monkeys (60 mg/kg/day in monkeys, 1152 mg/human as the HED), and is greater than the NOAEL in the 4-week toxicity study in rats (30 mg/kg/day in rats, 288 mg/human as the HED). The toxic findings observed in the nonclinical toxicity studies were vomiting, GI mucosal irritation, increased plasma hepatobiliary parameters with hepatocellular vacuolation, and histopathological lesions in the kidneys with increased plasma creatinine and urea nitrogen; however, these findings were not life-threatening or irreversible and can be monitored by general clinical exams. The gastric mucosal irritation, the most sensitive AE in the 4-week toxicity study in rats, was considered to be related to local exposure rather than systemic exposure; therefore, the planned maximum single dose in the SAD segment of the study is considered to be below the NOAEL in the 4-week toxicity study

in rats (1800 mg/human/day as human mg/kg equivalent dose assuming a body weight of approximately 60 kg in a healthy subject). Study participants will be closely monitored for events prior to escalating doses; therefore, it is justifiable to dose study participants up to 1000 mg in this study.

The MAD segment of the study will evaluate QD dosing of S-648414 for 14 days. The initial planned dose in the MAD segment of the study is 50 mg/day and the subsequent dose is 30 mg/day. Administration of 50 mg/day in the multiple-dose cohort will begin after review of safety data obtained from the 1000 mg/day single-dose cohort. If safety concerns are raised in the single-dose portion, the doses for MAD segment may be modified.

Part 3 of the study will evaluate the drug-interaction between S-648414 and dolutegravir. The treatment schedule, including the washout period, are defined based on the terminal elimination half-life of dolutegravir (approximately 14 hours) (see dolutegravir package insert [11]) and S-648414 (expected to be < 24 hours). The dose of dolutegravir to be used in this study is set in accordance with the approved dosage and administration of dolutegravir (see dolutegravir package insert [11]). If safety concerns are raised in the single-dose portion, the doses for Part 3 may be modified.

After reviewing unblinded safety data from the 50 mg multiple dose group, it was determined that it is acceptable to increase the dose to 100 mg. The initial planned dose in Part 3 is 100 mg/day and the subsequent dose is 200 mg/day. Administration of 200 mg/day in the multiple-dose cohort will begin after review of safety data obtained from the 100 mg/day in Part 3.

4.4 End of Study Definition

A study participant is considered to have completed the study if he/she has completed all periods of the study including the last visit.

The end of the study is defined as the date of the last visit of the last study participant in the study.

5. STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1 Inclusion Criteria

Study participants are eligible to be included in the study only if all of the following criteria apply:

1. Male or female adults ≥ 18 years in USA or ≥ 20 years in Japan to ≤ 55 years of age, at the time of signing the informed consent form (ICF).
   a) Specific to Japan sites: enrollment in Part 3 (Group I and J) will consist of only White or Black or African American race.

2. Capable of giving signed informed consent as described in Section 10.1 (Appendix 1), which includes compliance with the requirements and restrictions listed in the ICF and in this protocol.
3. Body mass index (BMI) ≥ 18.5 to < 32.0 kg/m^2 at the Screening visit.
4. Considered medically healthy as determined by the investigator or subinvestigator (suitably qualified), based on medical history and clinical evaluations including physical examination, clinical laboratory tests, vital sign measurements, and 12-lead ECG at Screening and at upon admission to the CRU and prior to administration of study intervention on Day 1. Clinical evaluations during Screening can be repeated (for confirmatory purposes or if additional information is required to assess study participant's eligibility) at the discretion of the investigator; repeated procedures must be performed within the same Screening window.
5. Female study participants must not be a woman of childbearing potential (see Section 10.4 [Appendix 4]) and must either be postmenopausal (defined as no menses for 12 months without an alternative medical cause; follicle-stimulating hormone (FSH) to be tested for confirmation at Screening) or premenopausal with 1 of the following documented: hysterectomy, tubal ligation, bilateral salpingectomy, or bilateral oophorectomy.
6. Male study participants must agree to use contraception as detailed in Section 10.4 (Appendix 4) during the treatment period and for at least 3 months after the last dose of study intervention.

5.2 Exclusion Criteria

Study participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Considered by the investigator or subinvestigator (suitably qualified) to be ineligible for the study due to a history of or current condition of significant metabolic or endocrine, hepatic, renal, hematological, pulmonary, cardiovascular, GI, urological, immunological, neurological, or psychiatric disorders with clinical manifestations.
2. History or presence of cancer in last 5 years except for nonmelanoma skin cancers.
3. Risk factors for:
   a. Torsades de Pointes (eg, heart failure, cardiomyopathy, or family history of Long QT Syndrome or Brugada Syndrome)
   b. Unexplained syncope, sick sinus syndrome, second- or third-degree atrioventricular (AV) block, myocardial infarction, pulmonary congestion, cardiac arrhythmia, angina, prolonged QT interval, or conduction abnormalities
4. History of GI surgery or disease including, but not limited to, gastric band/gastric resection and/or intestinal resection and/or duodenal disease (ie, celiac disease)

that may result in clinically significant malabsorption (except for an appendectomy).

5. History of hypersensitivity or severe side effects induced by a drug.
6. Any condition requiring medication and/or other treatment, such as dietary restriction and physical therapy.
7. History of significant multiple and/or severe allergic symptoms including food allergy (NOTE: Study participants with seasonal allergies may participate unless they have ongoing symptoms).

Prior/Concomitant Therapy

8. Used drugs or substances known to be inducers or inhibitors of cytochrome P450 enzymes and/or P-glycoprotein within 28 days prior to admission to the CRU.
9. Used prescription or over-the-counter (OTC) drugs, antacids, proton pump inhibitors, H2 antagonists, Chinese herbal medicines, oral cannabidiol, vitamins, minerals, herbal, and dietary supplements within 14 days prior to admission to the CRU.

Caffeine, Alcohol, Recreational Drugs, and Tobacco or Nicotine

10. Refuses to abstain from ingesting caffeine- or xanthine-containing products/medications (eg, coffee, tea, cola drinks, other caffeinated beverages, or chocolate) from 24 hours prior to admission to the CRU or refuses to refrain from consuming such products throughout the study (including Follow-up period).
11. Consumed alcohol or used alcohol-containing products within 72 hours prior to admission to the CRU or refuses to refrain from consuming such products throughout the study (including Follow-up period).
12. History of recreational drug use in the previous 6 months or has a history of problematic alcohol use (defined as study participants who regularly consume excessive amounts of alcohol, defined as > 3 glasses of alcoholic beverages per day (1 glass is approximately equivalent to: beer [284 mL/10 ounces (oz.)], wine [125 mL/4 oz.] or distilled spirits [25 mL/1 oz.]).
13. A positive drug or alcohol screen at the Screening visit or upon admission to the CRU.
14. Used tobacco- or nicotine-containing products (including cigarette, pipe, cigar, chewing, nicotine patch, or nicotine gum) within 6 months prior to admission to the CRU or refuses to refrain from using tobacco- or nicotine-containing products throughout the study (including Follow-up period).

Meals and Dietary Restrictions

15. Consumed grapefruit, grapefruit juice, Seville orange juice, orange juice, apple juice, vegetables from the mustard green family (eg, kale, broccoli, watercress, collard greens, kohlrabi, Brussels sprouts, mustard greens), or charbroiled meats within 7 days prior to admission to the CRU or refuses to refrain from consuming such products throughout the study (including Follow-up period).

Diagnostic Assessments

16. A corrected QT (QTc) interval of > 450 msec for males and > 470 msec for females (Fridericia's method) at the Screening visit or upon admission to the CRU.
17. Systolic blood pressure is outside the range of 90 to 140 mm Hg, diastolic blood pressure is outside the range of 50 to 90 mm Hg, or pulse rate is outside the range of 40 to 100 beats per minute (bpm) or considered ineligible by the investigator or subinvestigator at the Screening visit or upon admission to the CRU.
18. Total bilirubin, alanine aminotransferase (ALT), or aspartate aminotransferase (AST) values are greater than the upper limit of normal (ULN) or estimated glomerular filtration rate (eGFR) < 90 mL/min/1.73 m^2 at Screening or upon admission to the CRU.
19. A positive serological test for untreated syphilis, positive hepatitis B surface antigen, positive hepatitis C virus antibody, or positive human immunodeficiency virus (HIV) antigen/antibody result at the Screening visit.

Prior/Concurrent Clinical Study Experience

20. Participated in any other investigational trials or has been exposed to other investigational drugs within 28 days or 5 half-lives of the previously administered investigational drug (date derived from last study procedure [blood collection or dosing] of previous trial), whichever is longer, prior to admission to the CRU.
21. Previously received S-648414.

Other Exclusions

22. Poor venous access.
23. Donated blood (> 500 mL) or had significant blood loss within 56 days of study admission to the CRU or donated plasma within 7 days prior to until admission to the CRU.
24. Considered inappropriate for participation in the study for any reason by the investigator or subinvestigator.

5.3 Lifestyle Considerations

Lifestyle considerations (including use of caffeine, alcohol, recreational drugs, and tobacco or nicotine and meals and dietary restriction) are included in the exclusions in Section 5.2.

5.3.1 Meals and Dietary Restrictions

Meals and dietary restrictions are described in exclusion criterion 15 and further discussed below:

1. Water will be allowed at all times except for 1 hour prior to administration of study intervention. See Table 6-1 for water requirements during administration of study intervention.

2. During site stay, all foods/beverages will be provided by the medical institution and study participants in the same treatment step (see Table 6-3) will be given the same meals (2000 to 2500 kcal per day, except for the days of admission and administration of study intervention) except for Step 3-2. From consent acquisition to the completion of the end-of-study (or early termination) examination, study participants must refrain from excess eating and excess drinking.
3. In each treatment step (see Table 6-3), except for Step 3-2, Steps 7, 8, 9, and 10, study participants will fast from all food and beverages, except water, for at least 10 hours prior to administration of study intervention. No food should be allowed for at least 4 hours postdose.
4. Study participants in Step 3-2 will fast from all food and beverages, except water, for at least 10 hours prior to consuming a high-fat, high-calorie breakfast. Study participants should be encouraged to consume the entire meal and within ≤ 30 minutes of starting. Study intervention will be administered 30 minutes after the start of the consumption of the meal. The breakfast will consist of a high-fat (approximately 50% of total caloric content of the meal) and high-calorie (approximately 800 to 1000 calories) meal with approximately 150, 250, and 500 to 600 calories from protein, carbohydrate, and fat, respectively (eg, 2 eggs fried in butter, 2 strips of bacon, 2 slices of toast with butter, 4 oz. of hash brown potatoes, and 8 oz. of whole milk). No food should be allowed for at least 4 hours postdose.
5. Study participants in Steps 7, 8, 9, and 10 will receive study intervention 30 minutes following consumption of a standardized breakfast. No food should be allowed for at least 4 hours postdose.

Caffeine, alcohol, recreational drugs, and tobacco or nicotine restrictions are described in exclusion criteria 10 through 14.

5.3.2 Activity

Study participants will be instructed to refrain from strenuous physical activity that could cause muscle aches or injury, including contact sports or heavy lifting, at any time from the Screening visit and throughout the study until the last outpatient visit.

5.4 Screen Failures

Screen failures are defined as study participants who consent to participate in the clinical study but are not subsequently exposed to study intervention (S-648414 or placebo). A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

6. STUDY INTERVENTION

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to, or used by, a study participant according to the study protocol.

The study interventions in this study are summarized in Table 6-1 and the number of tablets per dose group is summarized in Table 6-2.

6.1 Study Intervention(s) Administered

Table 6-1 Study Intervention(s)

Study Intervention Name:	S-648414	Placebo	Midazolam	Dolutegravir[a]
Dosage formulation:	Tablet	Tablet	Oral suspension	Tablet
Unit dose strength(s)/Dosage level(s):	Unit dose: 10 mg and 100 mg Dosage levels: 10 mg, 30 mg, 50 mg, 100 mg, 200 mg, 250 mg, 500 mg, 1000 mg	Unit dose: 10 mg and 100 mg Dosage levels: 10 mg, 30 mg, 50 mg, 100 mg, 250 mg, 500 mg, 1000 mg	Unit dose: 2 mg/mL) Dosage level: 5 mg	50 mg
Physical description:	10 mg tablets: 6 mm white round tablets 100 mg tablets: 9 mm white round tablets	10 mg tablets: 6 mm white round tablets 100 mg tablets: 9 mm white round tablets	Red or pinkish oral solution	Yellow round tablets
Route of administration:	Oral	Oral	Oral	Oral
Dosing instructions:	Administer 240 mL water during administration Part 1: fasted or fed state Part 2: fed state Part 3: Periods 2 and 3 in fed state See Section 5.3.1 for timing of food See number of tablets per dose group in Table 6-2	Administer 240 mL water during administration Part 1: fasted or fed state Part 2: fed state See Section 5.3.1 for timing of food See number of tablets per dose group in Table 6-2	Fed state See Section 5.3.1 for timing of food	Administer 240 mL water during administration Part 3: Periods 1 and 3: fed state: See Section 5.3.1 for timing of food

Study Intervention Name:	S-648414	Placebo	Midazolam	Dolutegravir[a]
Packaging and labeling:	Provided in HDPE bottle with desiccant, 1 bottle per box. Each bottle and box will be labeled as required per country requirement.	Provided in HDPE bottle with desiccant, 1 bottle per box. Each bottle and box will be labeled as required per country requirement.	Provided in glass bottle	Provided in bottle with desiccant
Manufacturer:	Shionogi Pharma	Shionogi Pharma	Perrigo or generic equivalent	ViiV
Shelf Life:	Upon opening the bottle, tablets must be used within 7 days.	Upon opening the bottle, tablets must be used within 7 days.	As described on bottle.	As described on bottle.

HDPE = high-density polyethylene

a For further information on dolutegravir, refer to Package Insert [11] (https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Tivicay/pdf/TIVICAY-PI-PIL.PDF#page=1 and https://gskpro.com/content/dam/global/hcpportal/ja_JP/products-info/viiv/tivicay/tivicay.pdf)

Table 6-2 Number of Tablets (S-648414 or Placebo) per Dose Group

Dose (mg)	10 mg Tablet	100 mg Tablet
10	1	0
30	3	0
50	5	0
100	0	1
200	0	2
250	5	2
500	0	5
1000	0	10

6.2 Preparation/Handling/Storage/Accountability of Study Intervention

1. Study intervention should be kept at room temperature (15ºC to 30ºC).
2. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study intervention received and any discrepancies are reported and resolved before use of the study intervention.
3. Only study participants enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention. All study intervention must be stored in a secure, environmentally controlled, and

monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

4. The investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (ie, receipt, study participant dispensation, reconciliation, and final disposition records).
5. Final disposition of unused study intervention will be performed by the study site according to the site's standard operating procedure (SOP) upon written approval from the sponsor.

6.3 Measures to Minimize Bias: Randomization and Blinding

A randomized list will be produced by an unblinded statistician and provided to the unblinded site pharmacist, who will dispense study intervention (S-648414 or placebo) based on the provided list. Before the study is initiated, instructions will be provided to the study personnel.

Once a randomization number has been assigned, it must not be re-assigned.

Study intervention will be dispensed at the study visits summarized in the Schedule of Activities (Part 1 in Figure 1-2, Part 2 in Figure 1-3, and Part 3 in Figure 1-4 and Figure 1-5).

In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a study participant's treatment assignment is warranted. Study participant safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact the sponsor prior to unblinding a study participant's treatment assignment unless this could delay emergency treatment of the study participant. Once it is determined to unblind, the investigator should contact the site pharmacist who can break the blind. If a study participant's treatment assignment is unblinded, the sponsor must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation and electronic case report forms (eCRF) as applicable.

6.4 Study Intervention Compliance

Study intervention dosing will be observed and recorded in the eCRF. Any study intervention refused, or if the study participant vomits within 1 hour of dosing, will also be recorded in the eCRF. The time of vomiting in relation to dosing will be recorded.

6.5 Prior Therapy/Concomitant Therapy

Participants must abstain from taking prescription or OTC drugs (including vitamins and dietary or herbal supplements) within 14 days before the start of first dose of study intervention (S-648414 or placebo) until completion of the Follow-up visit.

In addition, from 28 days prior to admission to the completion of the study (including Follow-up period) the use of drugs or substances known to be inducers or inhibitors of cytochrome P450 enzymes and/or P-glycoprotein are prohibited.

Concomitant therapies and medical procedures are prohibited during the study unless they are essential for the treatment of an AE:

- From 14 days prior to admission to the completion of the study (including Follow-up period): the use of any therapies (prescribed or OTC drugs; antacids; proton pump inhibitors; H2 antagonists; and Chinese herbal medicines, oral cannabidiol, vitamins, minerals, herbal, and dietary supplements that have therapeutic effects) except for the study intervention (S-648414 or placebo).
- During the study (admission to the completion of the study, including Follow-up period): medical procedures are prohibited unless essential for treatment of an AE.

The medical monitor should be contacted if there are any questions regarding prior or concomitant therapy.

6.6 Dose Modification of Study Intervention

The sequence of steps in Part 1 (SAD), Part 2 (MAD) and Part 3 (MAD with dolutegravir) is summarized in Table 6-3.

Table 6-3 Sequence of Steps

Group	Sequence										
Part 1 – Single dosing after overnight fast of ≥ 10 hours											
Group A-1[a] (10 mg)	Step 1-1										
Group A-2[a] (10 mg)		Step 1-2									
Group B (30 mg)			Step 2								
Group C[b] (100 mg)				Step 3-1 Fasted	Step 3-2 Fed (30 minutes after the initiation of breakfast)						
Group D (250 mg)					Step 4						
Group E (500 mg)						Step 5					
Group F (1000 mg)							Step 6				
Part 2 – Multiple dosing administered 30 minutes after the initiation of breakfast											
Group G (50 mg)								Step 7[c] MDZ			
Group H (30 mg)									Step 8[c] MDZ		
Part 3 – Multiple dosing administered 30 minutes after the initiation of breakfast											
Group I (100 mg)										Step 9[d]	
Group J (200 mg)											Step 10[d]

MDZ = midazolam

a The initial dose group (Group A) is divided into Group A-1 including 2 study participants (1 S-648414 and 1 placebo) and Group A-2 including 6 study participants (5 S-648414 and 1 placebo). Study participants in Group A-2 will receive study intervention (S-648414 or placebo) in a sentinel dosing sequence, ie, only after confirmation that there are no clinical concerns in Group A-1 within 24 hours postdose.

b Group C-1 is the same as Step 3-1, and will be followed sequentially by Group C-2 (Step 3-2). For Group C, study participants will receive the first dose of study intervention (100 mg) in a fasted state (Group C-1), followed by a ≥ 14 days washout period and then the second dose of study intervention (100 mg) in a fed state (Group C-2).

c Steps 7 and 8 will include administration of MDZ on Day -2 and Day 14 and multiple administrations of S-648414 or matching placebo.

d Steps 9 and 10 will include administration of dolutegravir 50 mg once daily for 7 days, followed by a washout period of at least 7 days, followed by administration of S-648414 for 7 days, followed by coadministration of S-648414 and dolutegravir 50 mg once daily for 7 days.

6.6.1 Part 1 – Single Dosing

In Groups A, B, D, E, and F, eligible healthy adult study participants will be assigned to 1 of 5 ascending dose groups (10 to 1000 mg) and receive a single oral dose on Day 1 of S -648414 or placebo in a fasted state with 6 study participants per dose group receiving active drug and 2 study participants per dose group receiving placebo (see Table 4-1). Administration initiates from the lowest dose group and the next group does not initiate until the Follow-up period has been completed for the previous group except for A-2.

In Group C, 8 study participants will be assigned to S-648414 and 2 study participants assigned to placebo. Study participants assigned to the 100-mg group will receive a single oral dose of S-648414 in a fasted state (Group C-1), followed by a single dose of S-648414 in a fed state (high-fat meal) (Group C-2). Each episode of dosing will be separated by a washout period of at least 14 days.

The doses may be changed depending on the results of the safety, tolerability, and PK in the preceding group(s), if necessary. The highest dose will not exceed 1000 mg.

The investigator **and** the sponsor will evaluate the results of the safety and tolerability in the preceding group(s) and decide whether to proceed to the next dose group.

The next dose group will not be conducted if any of the following criteria are met:

- Any study participant experiences a treatment-related SAE (ie, treatment-emergent SAE considered related to the study intervention [S-648414 or placebo] by the investigator and sponsor)
- ≥ 50% of study participants experience grade 2 to 4 treatment-related AEs
- ≥ 25% of study participants experience grade 3 or 4 treatment-related AEs

6.6.2 Part 2 – Multiple Dosing

In Groups G and H, all study participants will receive a single oral dose of midazolam 5 mg alone on Day -2 and Day 14. In Group G, 8 study participants will be assigned to S-648414 (50 mg QD from Day 1 to Day 14) and 2 study participants to placebo (daily from Day 1 to Day 14). In Group H, 8 study participants will be assigned to S-648414 (30 mg QD from Day 1 to Day 14) and 2 study participants to placebo (daily from Day 1 to Day 14). Study participants will receive study intervention (S-648414 or placebo) 30 minutes after the initiation of breakfast.

The doses, mode of administration, frequency (ie, QD or BID), dietary condition (ie, fasted or fed), or length of administration may be changed depending on the results of the safety, tolerability, and PK in the preceding group(s), if necessary.

The investigator **and** the sponsor will evaluate the results of the safety and tolerability in the preceding group(s) and decide whether to proceed to the next dose group.

The next dose group will not be conducted if any of the following criteria are met:

- Any study participant experiences a treatment-related SAE (ie, treatment-emergent SAE considered related to the study intervention [S-648414 or placebo] by the investigator and sponsor)
- ≥ 50% of study participants experience grade 2 to 4 treatment-related AEs
- ≥ 25% of study participants experience grade 3 or 4 treatment-related AEs

6.6.3 Part 3 – Coadministration with Dolutegravir

In Groups I and J, study participants will receive dolutegravir (50 mg orally QD for 7 days) during Period 1, followed by no treatment for at least 7 days, followed by S-648414 for 7 days during Period 2, followed by coadministration of S-648414 and dolutegravir 50 mg QD for 7 days during Period 3.

Group I will receive 100 mg of S-648414 and Group J will receive 200 mg of S-648414. A total of 14 healthy adult study participants will be enrolled in Group I of Part 3; an additional 14 healthy adult study participants will be enrolled in Group J of Part 3.

The dose, mode of administration, frequency (ie, QD or twice daily [BID]), dietary condition (ie, fasted or fed), or length of administration may be changed, if necessary, by the sponsor depending on the results of the safety, tolerability, and PK in the preceding group(s).

The investigator **and** the sponsor will evaluate the results of the safety and tolerability in the preceding group(s) and decide whether to proceed to the next dose group.

The next dose group will not be conducted if any of the following criteria are met:

- Any study participant experiences a treatment-related SAE (ie, treatment-emergent SAE considered related to S-648414 by the investigator and sponsor)
- ≥ 50% of study participants experience grade 2 to 4 treatment-related AEs
- ≥ 25% of study participants experience grade 3 or 4 treatment-related AEs

See Section 10.3 (Appendix 3) for definitions of AEs, SAEs, assessment of intensity (including HIV grading system), assessment of causality, and procedures for recording, evaluating, follow-up, and reporting.

6.7 Intervention After the End of the Study

This is a Phase 1 study; therefore, there is no study intervention following the end of the study.

7. DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Part 1 is single dosing; therefore, discontinuation of study intervention is not applicable.

Part 2 and Part 3 are multiple dosing; therefore, the following applies only to Part 2 and Part 3:

Study participants will be discontinued from study intervention if the study participant requests to discontinue study intervention, but should be encouraged to remain in study for safety monitoring. If a study participant withdraws consent from any further intervention in the study, they will be discontinued.

See the Schedule of Activities for Part 2 (Figure 1-3) and for Part 3 (Figure 1-4 and Figure 1-5) for data to be collected at the time of discontinuation of study intervention and follow-up and for any further evaluations that need to be completed.

If a study participant discontinues from the study during Part 3, a replacement study participant may be enrolled if deemed appropriate by the sponsor.

7.1.1 Discontinuation of Study Intervention for Abnormal Liver Function

Discontinuation of study intervention for abnormal liver function should be considered by the investigator when a study participant meets 1 of the following conditions:

- ALT ≥ 3 x ULN
- The investigator believes that it is in best interest of the study participant
- If ALT ≥ 3 x ULN and bilirubin ≥ 2 x ULN (> 35% direct bilirubin) or INR > 1.5, report as a SAE (see Section 10.6 [Appendix 6])

Further details on liver chemistry stopping criteria and follow-up assessments can be found in Section 10.6 (Appendix 6).

7.1.2 Discontinuation of Study Intervention for Cardiac Changes

If a clinically significant finding is identified (including, but not limited to changes from baseline in QT interval corrected using Fridericia's formula [QTcF]) after enrollment, the investigator or qualified designee will determine if the participant can continue in the study and if any change in participant management is needed. This review of the ECG printed at the time of collection must be documented. Any new clinically relevant finding should be reported as an AE.

7.1.3 Discontinuation of Study Intervention for Pregnancy

As discussed in Section 8.3.5, if a female study participant becomes pregnant during the study, the investigator (or subinvestigator) will immediately discontinue study

intervention. Further details on collection of pregnancy information are summarized in Section 10.4 (Appendix 4).

7.2 Study Participant Discontinuation/Withdrawal from the Study

A study participant may withdraw from the study at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons. However, unless there is a safety reason, the study participant should remain in the trial even while off drug to protect the integrity of the study.

If the study participant withdraws consent, the sponsor may retain and continue to use any data collected before the withdrawal of consent.

If a study participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

See the Schedule of Activities (Part 1 in Figure 1-2, Part 2 in Figure 1-3, and Part 3 in Figure 1-4 and Figure 1-5) for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

7.3 Lost to Follow-up

A study participant will be considered lost to follow-up if he or she fails to return for scheduled visits and is unable to be contacted by the site staff.

The following actions must be taken if a study participant fails to return to the clinic for a required study visit:

- The site staff must attempt to contact the study participant and reschedule the missed visit as soon as possible, counsel the study participant on the importance of maintaining the assigned visit schedule, and ascertain whether or not the study participant wishes to and/or should continue in the study.
- Before a study participant is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the study participant by phone and the contact attempts should be documented in the study participant's medical record.
- Should the study participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

8. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the Schedule of Activities (Part 1 in Figure 1-2, Part 2 in Figure 1-3, and Part 3 in Figure 1-4 and Figure 1-5).
- Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.
- Adherence to the study design requirements, including those specified in the Schedule of Activities, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential study participants meet all eligibility criteria. The investigator must maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable (see Section 5.4).

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

8.1 Efficacy Assessments

Not applicable.

8.2 Safety Assessments

Planned time points for all safety assessments are provided in the Schedule of Activities. (Part 1 in Figure 1-2, Part 2 in Figure 1-3, and Part 3 in Figure 1-4 and Figure 1-5). Unless otherwise stated in the Schedule of Activities (Part 1 in Figure 1-2, Part 2 in Figure 1-3, and Part 3 in Figure 1-4 and Figure 1-5), predose procedures will be completed within approximately 1 hour of dosing and approximately ± 10 minutes for all postdose procedures

For any abnormal laboratory test results (hematology, blood chemistry, or urinalysis) or other safety assessments (eg, physical examination, ophthalmological examination, vital signs, ECGs) that worsen following exposure to the study intervention from baseline, the investigator will consider whether those results are clinically significant. Abnormal laboratory (including urine) test results are defined as values outside the reference range. For test results that are abnormal at baseline and significantly worsen following the initiation of the study, the investigator must also consider whether those results are clinically significant. Any test results that are considered clinically significant by the investigator are to be recorded as AEs. If the abnormal laboratory finding is associated with disease or organ toxicity, the investigator should report only the disease or organ toxicity as an AE.

The investigator will consider test results to be clinically significant in the following circumstances (at their own discretion in the other circumstances):

- Test results that lead to any of the outcomes included in the definition of an SAE (See Section 8.3).
- Test results that lead to a change in study intervention dosing or discontinuation from the study.
- Test results that lead to a concomitant drug treatment or other therapy.
- Test results that require additional diagnostic testing (except for a confirmatory test) or other medical intervention.
- Test results that meet the management and discontinuation criteria for abnormal liver function tests identified in Section 7.1 and Section 10.6 (Appendix 6).

In addition, when any test result meets the management and discontinuation criteria for liver function abnormalities (Section 7.1 and Section 10.6 [Appendix 6]), the results of further assessments and required follow-up should be recorded in the Liver Event Form.

8.2.1 Physical Examinations

- A complete physical examination will include, at a minimum, assessments of the cardiovascular, respiratory, GI, and neurological systems. Height and weight will also be measured and recorded. Complete physical examinations will be performed at visits specified in Section 1.3.
- A symptom-focused physical examination relevant to the study participant's current condition will be performed as clinically indicated at visits specified in Section 1.3 for new or worsening adverse events/symptoms.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

8.2.2 Vital Signs

- Body temperature, pulse rate, respiratory rate, and blood pressure will be assessed.
- Blood pressure and pulse measurements will be assessed in the supine position, with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (eg, television, cell phones). This needs to be documented in source notes.
- Vital signs (to be taken before blood collection for laboratory tests) will consist of 1 pulse and 3 blood pressure measurements (3 consecutive blood pressure readings will be recorded at intervals of at least 1 minute). Each of the 3 blood pressure readings will be recorded on the eCRF.

8.2.3 Electrocardiograms

- A triplicate 12-lead ECG for safety assessment will be obtained as outlined in the Schedule of Activities (see Section 1.3) using an ECG machine that automatically

calculates the heart rate (HR) and measures PR, QRS, QT, and QTc intervals. Refer to Section 7.1.2 for QTc withdrawal criteria and any additional QTc readings that may be necessary. At each time point at which triplicate ECGs are required, 3 individual ECG tracings should be obtained as closely as possible in succession, but no more than 2 minutes apart. The full set of triplicates should be completed in less than 4 minutes.

- High-precision 12-lead ECG tracings for cardiodynamic ECG evaluation will be extracted from the Holter recording as outlined in the Schedule of Activities (see Section 1.3) and analyzed according to the procedures and algorithm of the ECG vendor Core Laboratory. Electrocardiograms will be recorded after vital signs and prior to blood draws. Study participants will rest for 10 minutes in a supine position before and during the vital signs, ECG recording, and blood draws and the rest must be documented in source notes. The Holter equipment will be supplied and supported by ERT. All ECG data will be collected using a Global Instrumentation (Manlius, NY, USA) M12R ECG continuous 12-lead digital recorder. The continuous 12-lead digital ECG data will be stored on secure digital memory cards.

The following will be applied in ERT's core laboratory:

- ECG analysts are blinded to the study participant, visit and study intervention allocation.
- Baseline and on-treatment ECGs for a particular study participant will be over-read on the same lead and will be analyzed by the same reader.
- The primary analysis lead is Lead II. If Lead II is not analyzable, then primary lead of analysis will be changed to another lead for the entire study participant data set.

The following is a brief description of ECG analysis methods utilized by ERT's core laboratory.

8.2.3.1 TQT Plus Electrocardiogram Extraction Technique

Ten 14-second digital 12-lead ECG tracings will be extracted from the continuous Holter recordings using the "TQT Plus method", a computer-assisted and statistical process utilized by ERT. The method enables extraction of ECGs with the lowest HR variability and noise within the protocol-specified extraction time window (eg, the HR and QT changes from beat-to-beat in the range of < 10%). At each protocol-specified time point, 10 ECG replicates will be extracted from a 5-minute "ECG window" (typically, the last 5 minutes of the 15-minute period when the study participant is maintained in a supine position).

8.2.3.2 Expert-Precision QT Analysis

Expert-precision QT analysis will be performed on all analyzable (nonartifact) beats in the 10 ECG replicates. Statistical quality control (QC) procedures are 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

Measurements of all primary ECG parameters (QT, QTc, RR) in all recorded beats of all replicates that are deemed "high confidence" are performed using COMPAS software. All low confidence beats are reviewed manually and adjudicated using pass-fail criteria. The final QC assessment is performed by a cardiac safety specialist. The beats found acceptable by manual review are included in the analysis. The median QT, QTc, and RR value from each extracted replicate is calculated, and then the mean of all available medians from a nominal time point is used as the study participant's reportable value at that time point.

Categorical T-wave morphology analysis and the measurement of PR and QRS intervals will be performed manually in 3 of the 10 ECG replicates at each time point. Each fiducial point (onset of P-wave, onset of Q-wave, offset of S-wave, and offset of T-wave) is electronically marked.

For T-wave morphology and U-wave presence, treatment-emergent changes will be assessed, ie, changes not present at baseline. For each category of T-wave morphology and of U-waves, the category will be deemed as present if observed in all replicates at the time point. For baseline, the category will be deemed as present if observed in any replicates from all time points that constitute baseline. The T-wave morphology categories are described as follows.

Table 8-1 T-wave Morphology and U-wave Presence Categories (Assessed Manually)

Category	Description
Normal T-wave	Any positive T-wave not meeting any criterion below
Flat T-wave	T amplitude < 1 mm (either positive or negative) including flat isoelectric line
Notched T-wave (+)	Presence of notch(es) of at least 0.05 mV amplitude on ascending or descending arm of the positive T-wave
Biphasic	T-wave that contains a second component with an opposite phase that is at least 0.1 mV deep (both positive/negative and negative/positive and polyphasic T-waves included)
Normal T-wave (-)	T amplitude that is negative, without biphasic T-wave or notches
Notched T-wave (-)	Presence of notch(es) of at least 0.05 mV amplitude on descending or ascending arm of the negative T-wave
U-waves	Presence of abnormal U-waves

8.2.4 Clinical Safety Laboratory Assessments

- See Section 10.2 (Appendix 2) for the list of clinical laboratory tests to be performed and the Schedule of Activities (Part 1 in Figure 1-2, Part 2 in Figure 1-3, and Part 3 in Figure 1-4 and Figure 1-5) for the timing and frequency.
- The investigator must review the laboratory test results, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. The laboratory reports must be filed with the source documents.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or medical monitor.
    - If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the sponsor notified.
    - All protocol-required laboratory assessments, as defined in Section 10.2 (Appendix 2), must be conducted in accordance with the applicable local laboratory SOPs and the Schedule of Activities (Part 1 in Figure 1-2, Part 2 in Figure 1-3, and Part 3 in Figure 1-4 and Figure 1-5).
    - If laboratory values from nonprotocol-specified laboratory assessments performed at the institution's local laboratory require a change in participant management or are considered clinically significant by the investigator (eg, an SAE or AE or dose modification), then the results must be recorded in the eCRF.

8.2.5 Ophthalmological Examinations

- Ophthalmological examinations will include fundoscopy, tonometry (intraocular pressure), and a visual acuity test. Assessments will be carried out by an ophthalmologist at the specified time points in the Schedule of Activities (Part 1 in Figure 1-2, Part 2 in Figure 1-3, and Part 3 in Figure 1-4 and Figure 1-5).
- The investigator must review the ophthalmological results, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. Clinically significant abnormal findings would include new conditions detected or diagnosed after study intervention administration even though they may have been present before the start of the study (see Section 10.3 [Appendix 3]). The examinations must be filed with the source documents.
- All ophthalmological results with values considered clinically significantly abnormal during participation in the study should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or medical monitor. If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the sponsor notified.

8.3 Adverse Events and Serious Adverse Events

The definitions of an AE or SAE can be found in Section 10.3 (Appendix 3). The assessment of intensity (including HIV grading system), assessment of causality, and procedures for recording, evaluating, follow-up and reporting of AEs are also summarized in Section 10.3 (Appendix 3).

Adverse events reported by the study participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative) must be captured in source documents.

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study intervention and/or study procedures, or that caused the participant to discontinue the study intervention (see Section 7).

8.3.1 Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information

All AEs/SAEs will be collected from the date of signing of the ICF to up to 10 days after the last dose of study intervention or last Follow-up visit.

All SAEs will be recorded in the eCRF and reported to the sponsor or designee within 24 hours, as indicated in Section 10.3 (Appendix 3). The investigator or qualified designee will submit any updated SAE information to the sponsor within 24 hours of awareness.

Investigators or qualified designees are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the investigator or qualified designee learns of any SAE, including a death, at any time after a participant is no longer in the study, and the investigator or qualified designee considers the event to be reasonably related to the study treatment or study participation, the investigator or qualified designee must promptly notify the sponsor via phone, e-mail, or fax (see Section 10.3 [Appendix 3]). Investigator assessment of causality must be included with all SAEs reported to the sponsor. Serious adverse events with missing investigator causality will be followed up by the CRO urgently until response is provided to the sponsor.

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Section 10.3 (Appendix 3).

8.3.2 Method of Detecting Adverse Events and Serious Adverse Events

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and nonleading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

8.3.3 Follow-up of Adverse Events and Serious Adverse Events

After the initial AE/SAE report, the investigator or qualified designee is required to proactively follow each participant at subsequent visits/contacts. All AEs/SAEs and non-serious AEs of special interest (as defined in Section 8.3.6), will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is given in Section 10.3 (Appendix 3).

8.3.4 Regulatory Reporting Requirements for Serious Adverse Events

- Prompt notification by the investigator (or qualified designee) to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs), and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information (eg, summary or listing of SAEs) from the sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate, according to local requirements.

8.3.5 Pregnancy

- Details of all pregnancies in female participants and, if indicated, female partners of male participants will be collected after the start of study intervention and until 3 months after the last dose.
- If a female study participant becomes pregnant during the study, the investigator (or subinvestigator) will immediately discontinue study intervention.
- If a pregnancy is reported, the investigator should inform the sponsor within 24 hours of learning of the pregnancy and should follow the procedures, which require utilization of the Pregnancy Form, as outlined in Section 10.4 (Appendix 4).
- Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, and ectopic pregnancy) are considered SAEs.
- The outcome of the pregnancy (ie, birth, miscarriage, abortion) should be followed by the investigator and must also be reported using the Pregnancy Form.

8.3.6 Adverse Events of Special Interest

Adverse events of special interest include GI AEs such as nausea, vomiting, diarrhea, etc. If a GI AE occurs, the electronic data capture (EDC) system will generate an additional case report form to collect further information regarding the event.

8.4 Special Situations – Abuse, Misuse, Overdose, and Medication Error

Abuse, misuse, overdose, or medication error of the study intervention (Special Situations, as defined below) must be reported to the sponsor (sponsor's medical monitor) via EDC using a Special Situations Report Form as soon as possible. If there are associated SAEs, the investigator (or qualified designee) must also complete and submit an SAE submission in EDC as well.

- Abuse - persistent or sporadic, intentional excessive use of a study intervention(s), which is accompanied by harmful physical or psychological effects.
- Misuse - intentional and inappropriate use of a study intervention(s) other than as directed or indicated at any dose.
- Overdose - intentional or unintentional intake of study intervention(s) in excess of the assigned dose in the protocol.
- Medication Error - any unintended error in the prescribing, dispensing or administration of a study intervention(s) (including intercepted error). Cases of study participants missing doses of study intervention(s) are not considered reportable as medication error.

8.4.1 Treatment of Overdose

For this study, any dose of study intervention greater than that required by the phase of the protocol will be considered an overdose and reported as a special situation.

In the event of an overdose, the investigator should:

1. Contact the medical monitor immediately.
2. Closely monitor the participant for any AE/SAE and laboratory abnormalities until all AEs or abnormalities have resolved or are stable.
3. Obtain a plasma sample for PK analysis daily from the date of the last dose of study intervention if requested by the medical monitor (determined on a case-by-case basis).
4. Document the quantity of the excess dose as well as the duration of the overdose in the eCRF.

Decisions regarding dose interruptions or modifications will be made by the investigator in consultation with the medical monitor based on the clinical evaluation of the participant.

8.5 Pharmacokinetics

- In Groups A to F, plasma and urine samples of approximately 1 mL (each) will be collected for measurement of plasma and urine concentrations of S-648414 as specified in the Schedule of Activities (Part 1 in Figure 1-2). In Groups G and H, plasma and urine samples of approximately 1 mL (except for Days 14 and 15) and 2 mL (on Days 14 and 15) will be collected for measurement of plasma and urine concentrations of S-648414 or midazolam at each sampling point as specified in the Schedule of Activities (Part 2 in Figure 1-3). In Groups I and J, plasma samples of approximately 1 mL each will be collected for measurement of plasma concentrations of dolutegravir during Period 1 and S-648414 during Period 2, and 2 mL total will be collected for measurement of dolutegravir and S-648414 during Period 3 (on Days 22, 25 to 27, 28, and 29) at each sampling point as specified in the Schedule of Activities (Part 3 in Figure 1-4 and Figure 1-5). All samples will be stored at -65°C to -85°C and shipped on dry ice to the bioanalytical laboratory at [redacted]. Instructions for the collection and handling of biological samples will be provided by the sponsor. The actual date and time (24-hour clock time) of each sample will be recorded.
- Plasma (approximately 1 mL) and urine (approximately 20 mL) samples will be collected for exploratory metabolite analysis in Part 1 as specified in the Schedule of Activities (Part 1 in Figure 1-2). Instructions for the collection and handling of biological samples will be provided by the sponsor. The actual date and time (24-hour clock time) of each sample will be recorded. These plasma and urine samples will be stored at -65°C to -85°C and shipped on dry ice to [redacted].

[redacted]

Any remaining plasma and urine samples may be used for exploratory metabolite profiling of S-648414 or quantification of its metabolites. In case such studies are conducted, the study plan and results will be reported separately.

Drug concentration information that may unblind the study will not be reported to investigative sites or blinded personnel until the study has been unblinded.

Pharmacokinetic parameters and definitions are provided in Section 9.4.4.1.2. Further details will be provided in a separate statistical analysis plan (SAP).

8.6 Pharmacodynamics

Pharmacodynamic parameters are not evaluated in this study.

8.7 Genetics

Pharmacogenomics (PGx) testing is the study of variations of DNA and RNA characteristics as related to drug response or mechanisms of disease. Samples for the PGx testing are collected except when the Global Development Plan of the product states that sample collection for PGx testing is not required. The PGx testing will be conducted as necessary if at any time it appears that genes potentially related to PK, sensitivities of the product, or mechanisms of disease are identified or it appears that there is a potential unexpected or unexplained variation in handling or response to the product.

The objective of the PGx testing is to investigate a possible genetic relationship to handling or response to S-648414, or to advance understanding of disease biology. Study participants that agree to participate in the PGx testing will have a blood sample (7 mL) collected on Day -1 (in Part 1) (see Figure 1-2), Day -3 (in Part 2) (see Figure 1-3), and Day -1 (in Part 3) (see Figure 1-4). These study participants must voluntarily sign a PGx ICF prior to the PGx sample collection. Samples for the PGx testing will be collected and stored at -65°C to -85°C and shipped on dry ice to the DNA banking facility. A separate research protocol will be created to specify the details of the research plan and the site of the PGx analysis when a conduct of a PGx testing will be determined. Detailed procedures for sample collection, handling, labeling, storage, and shipping will be specified in a separate document. Further information regarding PGx testing is included in Section 10.5 (Appendix 5).

8.8 Biomarkers

Biomarkers (4β-hydroxycholesterol and cholesterol) in plasma will be measured as an enzyme activity marker. Biomarkers will be collected as specified in the Schedule of Activities (Part 2 in Figure 1-3). Samples will be stored at -65°C to -85°C and shipped on dry ice to [redacted].

The study plan and results will be reported separately.

8.9 Medical Resource Utilization and Health Economics

Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

9. STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

This is a Phase 1 dose-finding and food-effect study of the safety, tolerability, ventricular repolarization, PK of midazolam with and without S-648414, PK of dolutegravir with and without S-648414, and PK of S-648414 with and without dolutegravir; therefore, there is no formal statistical hypothesis in this study.

9.2 Sample Size Determination

Up to 50 healthy adult study participants in Part 1, 20 healthy adult study participants in Part 2, and 28 healthy adult study participants in Part 3 will be enrolled.

No formal calculations were performed to determine sample size for this study.

9.3 Populations for Analyses

For purposes of analysis, the following populations are defined:

Population	Description
Enrolled	All participants who sign the ICF.
Randomly Assigned to Study Intervention	All participants randomly assigned to study intervention.
Safety	All participants randomly assigned to study intervention and who take at least 1 dose of study intervention. Participants will be analyzed according to the intervention they actually received.
PK	The PK concentration population includes all study participants who receive at least 1 dose of S-648414 or dolutegravir and have at least 1 evaluable concentration in plasma or urine. This population will be used for the concentration listing. The PK parameter population includes all study participants with at least 1 PK parameter estimated appropriately. This population will be used for PK parameter listing and summary. This population will also be used for plotting of the concentration-time data, the concentration summary and statistical analysis.
QT/QTc	All study participants in the Safety population who have measurements at baseline as well as on-treatment, with at least 1 postdose time point with a valid ΔQTcF value. This analysis population will be used for the by-time point and categorical analyses for the cardiodynamic ECG parameters.
PK/QTc	All study participants who are in both the QT/QTc and PK populations with at least 1 pair of postdose plasma concentration and QTcF data from the same time point as well as study participants in the QT/QTc population who received placebo. This analysis population will be used for the concentration-QTc analysis.

ECG = electrocardiogram; ICF = informed consent form; PK = pharmacokinetic; QTcF = Fridericia's corrected QT; Δ = change from baseline

9.4 Statistical Analyses

The SAP will be finalized before unblinding/database lock. The SAP will describe the study participant populations to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. This section is a summary of the planned statistical analyses of the primary and secondary endpoints.

Unless otherwise noted, continuous variables will be summarized by using the number of nonmissing observations, arithmetic mean, standard deviation, median, minimum, and maximum values as descriptive statistics; categorical variables will be summarized by using the frequency count and the percentage of study participants in each category as descriptive statistics. All safety and demographic analyses described in the sections below will be performed for each part of the study.

All analyses and tabulations will be performed by using both SAS Version 9.2 or higher and WinNonlin Version 6.2.1 or higher.

9.4.1 Disposition

Among the study participants enrolled into the study, the number and percentage who complete the study and who prematurely discontinued the study will be summarized. In addition, reasons leading to study discontinuation will be summarized. The number of study participants included in each analysis population will also be presented.

9.4.2 Demographics, Treatment Compliance, and Prior and Concomitant Therapies

Demographic and baseline characteristics will be summarized with descriptive statistics for the safety population.

The study intervention exposure and compliance will be listed.

Prior therapies for drugs will be coded using the World Health Organization (WHO) Drug Dictionary. Study participants who have received prior therapy(ies) will be listed for the safety population.

Concomitant therapies for drugs will be coded using the WHO Drug Dictionary. Study participants who received concomitant therapy(ies) will be listed for the safety population.

9.4.3 Safety Analyses

All safety analyses will be performed on the Safety Population.

Endpoint	Statistical Analysis Methods
Adverse event	The number of study participants who experience at least 1 AE, death, other SAEs and AEs leading to study intervention (S-648414, midazolam, dolutegravir. or placebo) withdrawal will be counted for each treatment group. The number of AEs will also be presented. Treatment-related AEs will be summarized in the same manner as the overall summary of AEs. The number of study participants who experience at least 1 AE and incidence by system organ class and preferred term will be presented for each treatment group. The summary of AEs by severity will also be presented by system organ class and preferred term.
Cardiodynamic ECG evaluation	Summary statistics for each parameter and for the change from baseline to each time point will be calculated.
Clinical laboratory tests, vital signs, and ECGs	Summary statistics for each parameter and for the change from baseline to each time point will be calculated.
Physical examinations	Will be summarized by visit.

AE = adverse event; ECG = electrocardiogram; SAE = serious adverse event

9.4.4 Other Analyses

9.4.4.1 Pharmacokinetic Analyses

All PK analyses will be performed on the PK Population.

Endpoint	Statistical Analysis Methods
Primary	• To evaluate the effect of S-648414 on the pharmacokinetics of dolutegravir (Section 9.4.4.1.8) • To evaluate the effect of dolutegravir on the pharmacokinetics of S-648414 (Section 9.4.4.1.9)
Secondary	• Pharmacokinetic parameters (See Section 9.4.4.1.2 for statistical analyses). • Effect of high-fat meal (See Section 9.4.4.1.5 for statistical analyses). • Inhibition or induction of the CYP3A enzymes (See Section 9.4.4.1.7 for statistical analyses).

9.4.4.1.1 Plasma and Urine Concentration

Plasma concentrations of S-648414, midazolam, and dolutegravir will be listed and summarized by dosing regimen and nominal sampling time with the number of nonmissing observations (N), arithmetic mean (mean), standard deviation (SD) and coefficient of variation (CV%, calculated by SD/mean × 100), geometric mean and coefficient of variation for geometric mean (Geometric CV%), and median, minimum, and maximum values at each sampling time. The Geometric CV% will be calculated according to a formula Geometric CV% = $[\exp(sd^2)-1]^{1/2} \times 100$, where SD is the standard deviation for natural log (ln)-transformed data. Time course profiles for plasma

concentrations will be presented by appropriate graphics. In Part 2, achievement of steady state for plasma S-648414 concentration and in Part 3 achievement of steady state for plasma S-648414 and dolutegravir will be assessed by visual inspection of trough concentration data.

For summary of plasma concentration, plasma concentration below the lower limit of quantification (BLQ) will be treated as zero (0) for calculations of mean, SD, CV%, median, minimum, and maximum values and treated as missing for calculation of geometric mean and Geometric CV% mean values.

Urine volume and urine concentration data for S-648414 will be listed. In Part 1, time course profiles for cumulative fraction of dose excreted in urine will be presented by appropriate graphics.

9.4.4.1.2 Pharmacokinetic Parameters

The following PK parameters will be calculated, whenever possible, for S-648414, midazolam, and dolutegravir from plasma and urine concentration data by noncompartmental methods. Other parameters may be computed, as appropriate, upon review of the data. The estimated PK parameters will be computed for each study participant using the actual sample collection times recorded during the study.

Pharmacokinetic parameters (S-648414) for Part 1:

C_{max} (ng/mL)	Maximum plasma concentration
T_{max} (hr)	Time to maximum plasma concentration
$AUC_{0\text{-last}}$ (ng·hr/mL)	Area under the concentration-time curve from time zero to the time of the last quantifiable concentration after dosing, calculated by linear trapezoidal method when concentrations are increasing and by logarithmic trapezoidal method when concentrations are decreasing (Linear Up/Log Down Trapezoidal Method)
$AUC_{0\text{-inf}}$ (ng·hr/mL)	Area under the concentration-time curve extrapolated from time zero to infinity defined as $AUC_{0\text{-last}} + (C_{last}/\lambda_z)$, where C_{last} is the last measurable plasma concentration and λ_z is the plasma terminal elimination rate constant
$t_{1/2,z}$ (hr)	Terminal elimination half-life, where $t_{1/2,z} = (\ln 2)/\lambda_z$
λ_z (hr^{-1})	Terminal elimination rate constant, where λ_z is the magnitude of the slope of the linear regression of the log concentration versus time profile during the terminal phase
MRT (hr)	Mean residence time, where $MRT = AUMC_{0\text{-inf}}/AUC_{0\text{-inf}}$ and $AUMC_{0\text{-inf}}$ is the area under the first moment curve extrapolated to infinity
CL/F (L/hr)	Apparent total clearance estimated according to: $CL/F = Dose/AUC_{0\text{-inf}}$ for treatment groups

V_z/F (L)	Apparent volume of distribution in the terminal elimination phase, estimated according to: $V_z/F = Dose/AUC_{0\text{-}inf}/\lambda_z$
$Feu_{0\text{-}96}$ (%)	Fraction of dose excreted in urine from 0 to 96 hours; calculated as $Aeu_{0\text{-}96}/Dose \times 100$ and $Aeu_{0\text{-}96}$ is cumulative amount of the drug excreted in urine during a given collection time interval from 0 to 96 hours
CL_R (L/hr)	Renal clearance estimated according to: $CL_R = Aeu_{0\text{-}96}/AUC_{0\text{-}last}$

Pharmacokinetic parameters (S-648414) for Part 2:

C_{max} (ng/mL)	Maximum plasma concentration on Day 1 and Day 14
T_{max} (hr)	Time to maximum plasma concentration on Day 1 and Day 14
$AUC_{0\text{-}\tau}$ (ng·hr/mL)	Area under the concentration-time curve over the dosing interval τ (24 hours) on Day 1 and Day 14, calculated by Linear Up/Log Down Trapezoidal Method
$t_{1/2,z}$ (hr)	Terminal elimination half-life, where $t_{1/2,z} = (\ln 2)/\lambda_z$ on Day 14
λ_z (hr^{-1})	Terminal elimination rate constant, where λ_z is the magnitude of the slope of the linear regression of the log concentration versus time profile during the terminal phase on Day 14
CL/F (L/hr)	Apparent total clearance estimated according to: $CL/F = Dose/AUC_{0\text{-}\tau}$ on Day 14
V_z/F (L)	Apparent volume of distribution in the terminal elimination phase on Day 14, estimated according to: $V_z/F = Dose/AUC_{0\text{-}\tau}/\lambda_z$
$Feu_{0\text{-}\tau}$ (%)	Fraction of dose excreted in urine over the dosing interval τ (24 hours) on Day 14; calculated as $Aeu_{0\text{-}\tau}$ / Dose × 100, where $Aeu_{0\text{-}\tau}$ is the amount of drug excreted in urine
CL_R (L/hr)	Renal clearance on Day 14, calculated as $CL_R = Aeu_{0\text{-}\tau} / AUC_{0\text{-}\tau}$ and $Aeu_{0\text{-}\tau}$ is cumulative amount of the drug excreted in urine during a given collection time over the dosing interval τ (24 hours)

Pharmacokinetic parameters for midazolam on Day -2 and Day 14:

C_{max} (ng/mL)	Maximum plasma concentration
T_{max} (hr)	Time to maximum plasma concentration
$AUC_{0\text{-}last}$ (ng·hr/mL)	Area under the concentration-time curve from time zero to the time of the last quantifiable concentration after dosing, calculated by the Linear Up/Log Down Trapezoidal Method
$AUC_{0\text{-}inf}$ (ng·hr/mL)	Area under the concentration-time curve extrapolated from time zero to infinity defined as $AUC_{0\text{-}last} + (C_{last}/\lambda_z)$, where C_{last} is the last measurable plasma concentration and λ_z is the plasma terminal elimination rate constant

$t_{1/2,z}$ (hr)	Terminal elimination half-life, where $t_{1/2,z} = (\ln 2)/\lambda_z$
λ_z (hr^{-1})	Terminal elimination rate constant, where λ_z is the magnitude of the slope of the linear regression of the log concentration versus time profile during the terminal phase
MRT (hr)	Mean residence time, where MRT = $AUMC_{0\text{-inf}}/AUC_{0\text{-inf}}$ and $AUMC_{0\text{-inf}}$ is the area under the first moment curve extrapolated to infinity

Pharmacokinetic parameters (S-648414) for Part 3:

C_{max} (ng/mL)	Maximum plasma concentration on Day 21 and Day 28
T_{max} (hr)	Time to maximum plasma concentration on Day 21 and Day 28
C_τ (ng/mL)	Plasma concentration at the end of the dosing interval τ (24 hours) on Day 21 and Day 28
$AUC_{0\text{-}\tau}$ (ng·hr/mL)	Area under the concentration-time curve over the dosing interval τ (24 hours) on Day 21 and Day 28, calculated by Linear Up/Log Down Trapezoidal Method
CL/F (L/hr)	Apparent total clearance estimated according to: CL/F =Dose/$AUC_{0\text{-}\tau}$ on Day 21 and Day 28

Pharmacokinetic parameters (dolutegravir) for Part 3:

C_{max} (ng/mL)	Maximum plasma concentration on Day 7 and Day 28
T_{max} (hr)	Time to maximum plasma concentration on Day 7 and Day 28
C_τ (ng/mL)	Plasma concentration at the end of the dosing interval τ (24 hours) on Day 7 and Day 28
$AUC_{0\text{-}\tau}$ (ng·hr/mL)	Area under the concentration-time curve over the dosing interval τ (24 hours) on Day 7 and Day 28, calculated by Linear Up/Log Down Trapezoidal Method
CL/F (L/hr)	Apparent total clearance estimated according to: CL/F = Dose/$AUC_{0\text{-}\tau}$ on Day 7 and Day 28

The estimated PK parameters will be summarized by dosing regimen with N, mean, SD, CV%, geometric mean, Geometric CV%, median, minimum, and maximum values. The T_{max} will be summarized by analyte and dosing regimen with N, mean, SD, CV%, median, minimum, and maximum values. If the number of PK parameter data is < 3, the data will not be summarized.

If the number of data points used to calculate λ_z is < 3 or the calculated coefficient of determination (R^2) value for λ_z is < 0.800, then that study participant's λ_z, and $AUC_{0\text{-inf}}$, $t_{1/2,z}$, MRT, CL/F, and V_z/F derived from λ_z will be flagged in the data listing, and excluded from the descriptive and statistical analysis. If λ_z cannot be determined, then $AUC_{0\text{-inf}}$, $t_{1/2,z}$, MRT, CL/F, and V_z/F will not be estimated.

In addition to the parameters listed above, the extrapolated percent of $AUC_{0\text{-}inf}$, calculated as AUC_{extr} (%) = 100 × ($AUC_{0\text{-}inf}$ − $AUC_{0\text{-}last}$)/$AUC_{0\text{-}inf}$, will be determined. If the AUC_{extr} is greater than 20%, then $AUC_{0\text{-}inf}$, MRT, CL/F, and V_z/F derived from $AUC_{0\text{-}inf}$ will be flagged in the data listing and excluded from the descriptive statistics and statistical analysis.

Individual plasma concentrations, if deemed to be anomalous, may be excluded from the analysis at the discretion of the PK study director. Any such exclusion will be clearly represented in the study report along with justification for exclusion.

For the calculations of PK parameters, BLQ before the occurrence of the first quantifiable concentration will be treated as zero, and BLQ after the first occurrence of the quantifiable concentration will be treated as missing. Urine BLQ concentrations will be treated as 0 for calculation of $Aeu_{0\text{-}96}$ and $Aeu_{0\text{-}\tau}$.

Pharmacokinetic calculations will be performed by using WinNonlin Version 6.2.1 or higher.

9.4.4.1.3 Dose Proportionality

Dose proportionality of PK parameter of S-648414 will be examined in Part 1 except for Step 3-2 (Tablet Fed), and in Part 2. In Part 1, dose proportionality will be assessed for C_{max}, $AUC_{0\text{-}last}$, and $AUC_{0\text{-}inf}$ of S-648414 using the power model. The power model assumes a linear relationship between the ln-transformed parameter and ln-transformed dose.

$$\ln(\text{Parameter}) = \alpha + \beta \times \ln(\text{Dose}) + \text{Random error}$$

Where Parameter is a given PK parameter, α is the intercept, β is the slope, and Random error is a random residual error. Dose proportionality implies that slope = 1 and will be assessed by estimating mean slope with the corresponding confidence interval (95% CI) using SAS Proc Reg from the power model.

In the case where the 95% CI of the slope from the power model does not include 1, the differences in ln-transformed C_{max}, $AUC_{0\text{-}last}$, and $AUC_{0\text{-}inf}$ of S-648414 will be examined between treatment groups. An analysis of variance (ANOVA) will be performed using SAS Proc Mixed. An ANOVA will be also performed for Part 2. The point estimates and 90% CIs will be generated for the ratios of all treatment group combinations for ln-transformed C_{max} and AUC ($AUC_{0\text{-}last}$ and $AUC_{0\text{-}inf}$ for Part 1 and $AUC_{0\text{-}\tau}$ for Part 2). The point estimates and 90% CIs will be back-transformed to obtain the corresponding geometric least squares mean ratios and 90% CIs. The following linear model will be used:

$$\ln(\text{Parameter}) = \text{Treatment group} + \text{Random error}$$

Where Parameter is a given PK parameter, Treatment group is a fixed treatment group effect and Random error is a random residual error.

The relationship of C_{max}, $AUC_{0\text{-}last}$, and $AUC_{0\text{-}inf}$ to dose will be graphically represented.

Dose proportionality will be concluded based on the point estimates and CIs of the power model or the ANOVA model and visual inspection of the corresponding plots.

9.4.4.1.4 Dose Independency

Dose independence of PK parameters of S-648414 will be examined between the treatment groups in Part 1 except for Step 3-2 (Tablet Fed), and Part 2. For $t_{1/2,z}$, CL/F, V_z/F, MRT, and CL_R, the difference between treatment groups will be examined for dose independence using the ANOVA model. An ANOVA will be performed using SAS Proc Mixed. The point estimates and 90% CIs will be generated for all treatment group combinations for ln-transformed $t_{1/2,z}$, CL/F, V_z/F, MRT, and CL_R. The point estimates and 90% CIs will be back-transformed to obtain the corresponding geometric least squares mean ratios and 90% CIs. The following linear model will be used:

ln (Parameter) = Treatment group + Random error

Where Parameter is a given PK parameter, Treatment group is a fixed treatment group effect and Random error is a random residual error.

The relationship of $t_{1/2,z}$, CL/F, V_z/F, MRT, and CL_R to dose will be graphically represented.

Dose independency will be concluded based on the point estimates and 90% CIs of the ANOVA model and visual inspection of the corresponding plots.

9.4.4.1.5 Effect of Food

When data are available, the PK after administration of S-648414 tablet will be compared between the fasted state (Step 3-1) and the fed state (Step 3-2). An ANOVA will be performed using SAS Proc Mixed for ln-transformed C_{max}, $AUC_{0\text{-}last}$, $AUC_{0\text{-}inf}$, and $t_{1/2,z}$. In case of unbalanced data, the Kenward-Roger method will be used to compute the denominator degrees of freedom for the tests of fixed effects in this analysis. The point estimates and 90% CIs will be generated for the differences between PK in the fed state and PK in the fasted state for ln-transformed C_{max}, $AUC_{0\text{-}last}$, $AUC_{0\text{-}inf}$, and $t_{1/2,z}$. The point estimates and 90% CIs will be back-transformed to obtain the corresponding geometric least squares mean ratios and 90% CIs as the following ratio.

(PK in the fed state [Step 3-2]) / (PK in the fasted state [Step 3-1])

The following linear mixed effects model will be used:

ln (Parameter) = Food condition + Subject + Random error

Where Food condition is a fixed effect of food condition, Subject is a random effect of study participant, and Random error is a random residual error.

The comparison of C_{max}, T_{max}, $AUC_{0\text{-}last}$, $AUC_{0\text{-}inf}$, and $t_{1/2,z}$ between the fasted state and the fed state will be graphically represented.

The effect of food will be concluded based on the point estimates and 90% CIs of the ANOVA model and visual inspection of the corresponding plots.

9.4.4.1.6 Accumulation Ratio

When data are available, the accumulation ratios of S-648414 calculated as ratios of Day 14 to Day 1 will be assessed in Part 2. An ANOVA will be performed using SAS Proc Mixed for ln-transformed C_{max} and $AUC_{0\text{-}\tau}$. In case of unbalanced data, the Kenward-Roger method will be used to compute the denominator degrees of freedom for the tests of a fixed effect in the analysis. The point estimates and their 90% CIs will be generated for the differences between Days 1 and 14 for ln-transformed C_{max} and $AUC_{0\text{-}\tau}$ in each dose group. The point estimates and the 90% CIs will be back-transformed to obtain the corresponding geometric least squares mean ratios and the 90% CIs. The following linear mixed effects model will be used:

ln (Parameter) = Day + Subject + Random error

Where Day is a fixed effect of day, Subject is a random effect of study participant, and Random error is a random residual error.

9.4.4.1.7 Effect of S-648414 on the Pharmacokinetics of Midazolam

When data are available, the effect of S-648414 on the PK of midazolam will be assessed. An ANOVA will be performed using SAS Proc Mixed for ln-transformed C_{max}, $AUC_{0\text{-}last}$, and $AUC_{0\text{-}inf}$ of midazolam. In case of unbalanced data, the Kenward-Roger method will be used to compute the denominator degrees of freedom for the tests of a fixed effect in the analysis. The point estimates and their 90% CIs will be generated for the differences between midazolam coadministered with S-648414 and midazolam alone for ln-transformed C_{max}, $AUC_{0\text{-}last}$, and $AUC_{0\text{-}inf}$. The point estimates and their 90% CIs will be back-transformed to obtain the corresponding geometric least squares mean ratios and 90% CIs as the following ratio:

(midazolam coadministered with S-648414) / (midazolam alone)

The following linear mixed effects model will be used:

ln (Parameter) = Treatment + Subject + Random error

Where Treatment is a fixed effect of treatment, Subject is a random effect of study participant, and Random error is a random residual error.

The drug interaction will be assessed by whether the 90% CIs for C_{max}, $AUC_{0\text{-}last}$, and $AUC_{0\text{-}inf}$ of midazolam are completely contained within the range of 0.8000 to 1.2500.

The comparison of C_{max}, $AUC_{0\text{-last}}$, and $AUC_{0\text{-inf}}$ between midazolam alone and midazolam coadministered with S-648414 will be graphically represented.

The effect of S-648414 on the PK of midazolam will be concluded based on the point estimates and 90% CIs of the ANOVA model and visual inspection of the corresponding plots.

9.4.4.1.8 Effect of S-648414 on the Pharmacokinetics of Dolutegravir

When data are available, the effect of S-648414 on the PK of dolutegravir will be assessed. An ANOVA will be performed using SAS Proc Mixed for ln-transformed C_{max}, C_{τ}, and $AUC_{0\text{-}\tau}$ of dolutegravir. In case of unbalanced data, the Kenward-Roger method will be used to compute the denominator degrees of freedom for the tests of a fixed effect in the analysis. The point estimates and their 90% CIs will be generated for the differences between dolutegravir coadministered with S-648414 and dolutegravir alone for ln-transformed C_{max}, C_{τ}, and $AUC_{0\text{-}\tau}$. The point estimates and their 90% CIs will be back-transformed to obtain the corresponding geometric least squares mean ratios and 90% CIs as the following ratio:

(dolutegravir coadministered with S-648414) / (dolutegravir alone)

The following linear mixed effects model will be used:

ln (Parameter) = Treatment + Subject + Random error

Where Treatment is a fixed effect of treatment, Subject is a random effect of study participant, and Random error is a random residual error.

The drug interaction will be assessed by whether the 90% CIs for C_{max}, C_{τ}, and $AUC_{0\text{-}\tau}$ of dolutegravir are completely contained within the range of 0.8000 to 1.2500.

The comparison of C_{max}, C_{τ}, and $AUC_{0\text{-}\tau}$ between dolutegravir alone and dolutegravir coadministered with S-648414 will be graphically represented.

The effect of S-648414 on the PK of dolutegravir will be concluded based on the point estimates and 90% CIs of the ANOVA model and visual inspection of the corresponding plots.

9.4.4.1.9 Effect of Dolutegravir on the Pharmacokinetics of S-648414

When data are available, the effect of dolutegravir on the PK of S-648414 will be assessed. An ANOVA will be performed using SAS Proc Mixed for ln-transformed C_{max}, C_{τ}, and $AUC_{0\text{-}\tau}$ of S-648414. In case of unbalanced data, the Kenward-Roger method will be used to compute the denominator degrees of freedom for the tests of a fixed effect in the analysis. The point estimates and their 90% CIs will be generated for the differences between S-648414 coadministered with dolutegravir and S-648414 alone for ln-transformed C_{max}, C_{τ}, and $AUC_{0\text{-}\tau}$. The point estimates and their 90% CIs will be back-

transformed to obtain the corresponding geometric least squares mean ratios and 90% CIs as the following ratio:

(S-648414 coadministered with dolutegravir) / (S-648414 alone)

The following linear mixed effects model will be used:

ln (Parameter) = Treatment + Subject + Random error

Where Treatment is a fixed effect of treatment, Subject is a random effect of study participant, and Random error is a random residual error.

The drug interaction will be assessed by whether the 90% CIs for C_{max}, C_{τ}, and $AUC_{0-\tau}$ of S-648414 are completely contained within the range of 0.8000 to 1.2500.

The comparison of C_{max}, C_{τ}, and $AUC_{0-\tau}$ between S-648414 alone and S-648414 coadministered with dolutegravir will be graphically represented.

The effect of dolutegravir on the PK of S-648414 will be concluded based on the point estimates and 90% CIs of the ANOVA model and visual inspection of the corresponding plots.

9.4.4.2 Cardiodynamic Electrocardiogram Evaluation

When data are available, the relationship between S-648414 plasma concentrations and QT interval will be analyzed to evaluate the effect of S-648414 on the QTc interval using the Fridericia's method (QTcF). Details of the cardiodynamic ECG evaluation will be provided in the SAP. The results of cardiodynamic ECG evaluation will be reported as a separate report.

The primary analysis will be based on concentration-QTc modeling of the relationship between the concentrations of S-648414 and change-from-baseline QTcF (ΔQTcF) with the intent to exclude an effect > 10 msec at clinically relevant S-648414 plasma concentrations. In addition, the effect of S-648414 on the placebo-corrected ΔQTcF, ΔHR, ΔPR, and ΔQRS (ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS) will be evaluated at each postdosing time point ("by-time point" analysis) using the Intersection Union Test. An analysis of categorical outliers will be performed for changes in HR, PR, QRS, QTcF, T-wave morphology and U-wave presence.

9.5 Interim Analyses

No interim analyses for efficacy are planned in this study. However, ongoing assessments of the safety, tolerability, and PK will occur between the investigator and the sponsor. Criteria for determining whether to proceed to next dose group are included in Section 6.6.1 (Part 1) and Section 6.6.2 (Part 2). To assist with the evaluation and dose selection for the S-648414 Proof of Concept study, the sponsor will be unblinded after the completion of Part 2 and an evaluation of safety and PK data from Parts 1 and 2 will be conducted.

No independent data monitoring committee will be established for this study.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

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

10.1.1 Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Applicable ICH Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator's Brochure, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated. Competent authority notification, review, and approval may be required as appropriate according to local country requirements.
- Any amendments to the protocol will require competent authority and/or IRB/IEC approval (as appropriate) before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- For studies to be submitted as part of a European Marketing Authorization Application, the sponsor will select a Clinical Study Report Coordinating investigator who will sign the Clinical Study Report.
- The investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
  - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

10.1.2 Financial Disclosure

The information on financial disclosure for investigators will be addressed in a separate agreement between the sponsor and the investigator.

10.1.3 Informed Consent Process

- The investigator or his/her representative must explain the nature of the study to the study participant or his/her legally authorized representative and answer all questions regarding the study.
- Study participants must be informed that their participation is voluntary. Study participants will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, and Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the study participant was enrolled in the study and the date that written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Study participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the signed ICF(s) must be provided to the study participant or the study participant's legally authorized representative.

A participant who is rescreened is not required to sign another ICF if the rescreening occurs within 28 days from the previous ICF signature date.

A separate ICF will address the use of remaining mandatory samples for optional exploratory research. The investigator or authorized designee will explain to each study participant the objectives of the exploratory research. Study participants will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to document a participant's agreement to allow any remaining specimens to be used for exploratory research. Participants who decline to participate in this optional research will not provide this separate signature.

10.1.4 Data Protection

- Study participants will be assigned a unique identifier by the sponsor. Any study participant records or datasets that are transferred to the sponsor will contain the identifier only; study participant names or any information that would make the study participant directly identifiable will not be transferred.
- The study 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.
- The study participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5 Dissemination of Clinical Study Data

All information regarding S-648414 supplied by the sponsor to the investigator is privileged and confidential information. The investigator agrees to use this information to accomplish the study and will not use it for other purposes without consent from the sponsor. It is understood that there is an obligation to provide the sponsor with complete data obtained during the study. The information obtained from the clinical trial will be used toward the development of S-648414 and may be disclosed to regulatory authority(ies), other investigators, corporate partners, or consultants as required.

The sponsor will retain ownership of all data. All proposed publications based on the study will be subject to the sponsor's approval requirements.

10.1.6 Data Quality Assurance

- All study participant data relating to the study will be recorded on an eCRF unless transmitted to the sponsor or designee electronically (eg, laboratory data and central ECG data). The investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and sponsor and/or regulatory agency inspections and provide direct access to source data documents.
- The sponsor or designee is responsible for the data management of this study, including quality checking of the data.
- Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 25 years after study completion, unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.7 Source Documents

Source documents provide evidence of the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator’s site.

Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may

need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Source documents are defined as original documents, data, and records (eg, hospital records, clinical and office charts, laboratory notes, memoranda, study participant's diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, study participant files, and records kept at the pharmacy, laboratories, and medico-technical departments involved in the clinical trial).

10.1.8 Study and Site Closure

The sponsor/designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor.

Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected, data have been collected, a study-site closure visit has been performed, and notification to the IRB/IEC has been made.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor’s procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further study intervention development

10.2 Appendix 2: Clinical Laboratory Tests

- The tests detailed in Table 10-1 will be performed by the local laboratory.
- Protocol-specific requirements for inclusion or exclusion of study participants are detailed in Section 5 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 10-1 Protocol-required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	Platelet count RBC count Hemoglobin Hematocrit	RBC indices: MCV MCH % Reticulocytes		WBC count with differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils
Clinical chemistry[a]	Blood urea nitrogen	Potassium	AST	Total and direct bilirubin
	Creatinine	Sodium	ALT	Total protein
	Glucose fasting	Calcium	ALP	Total cholesterol
	Uric acid	Chloride	GGT	
	CRP	CPK	LDH	
Routine urinalysis[b]	• Specific gravity • pH, glucose, protein, blood, ketones, urobilinogen, bilirubin, nitrite, leukocyte esterase by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other screening tests	• Follicle-stimulating hormone and estradiol (as needed in women of nonchildbearing potential only) • Urine alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines) • Serology (syphilis, HIV antibody, hepatitis B surface antigen, and hepatitis C virus antibody)			

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CPK = creatine phosphokinase; CRP = C-reactive protein; GGT = gamma glutamyl transferase; HIV = human immunodeficiency virus; INR = international normalized ratio; IRB /IEC = Institutional Review Board/Independent Ethics Committee; LDH = lactate dehydrogenase; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; RBC = red blood cell; SAE = serious adverse event; ULN = upper limit of normal; WBC = white blood cell

a Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 7.1.1 and Section 10.6 (Appendix 6). All events of ALT ≥ 3 × ULN and bilirubin ≥ 2 × ULN (> 35% direct bilirubin) or ALT > 3 × ULN and INR > 1.5, if INR measured which may indicate severe liver injury (possible "Hy's Law") and must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis).

b Local urine testing will be standard for the protocol unless serum testing is required by local regulation or IRB/IEC.

All study-required laboratory assessments will be performed by a local laboratory.

Investigators must document their review of each laboratory safety report.

10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator. • Exacerbation of a chronic or intermittent preexisting condition including an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study intervention administration even though they may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events NOT Meeting the AE Definition
• The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Hospitalization for preplanned and elective procedures to treat a preexisting condition that did not worsen after start of study will not be considered an AE, and therefore will not be considered an SAE despite requiring hospitalization. The exception is when the patient experiences another event which is fatal, is life-threatening, results in disability, leads to prolonged hospitalization or is considered to be medically significant during/following the procedure. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of preexisting disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met.

A SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life threatening The term "life threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a preexisting condition that did not worsen from baseline is not considered an AE.
d. Results in persistent disability/incapacity • The term disability means a substantial disruption of a person's ability to conduct normal life functions. • This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle), which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e. Is a congenital anomaly/birth defect
f. Other situations: • Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Recording and Follow-up of AE and/or SAE

AE and SAE Recording
• When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event. • The investigator or qualified designee will then record all relevant AE/SAE information in the eCRF. • It is **not** acceptable for the investigator (or qualified designee) to send photocopies of the participant's medical records to the contract research organization (CRO) or sponsor in lieu of completion of the AE/SAE eCRF page.

- There may be instances when copies of medical records for certain cases are requested by the CRO or sponsor. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to the CRO or sponsor.
- The investigator or qualified designee will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
- In the case of a death, the cause of death should be reported instead of "Death" as death is an outcome and not the SAE.
- If the cause of death is unknown, death can be reported until the cause of death is determined, at which time the cause of death should be reported.

Assessment of Intensity

The investigator or qualified designee will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories (as per HIV grading system https://rsc.tech-res.com/docs/default-source/safety/daids-ae-grading-table-mar2017.pdf):

- Grade 1 (Mild): Mild symptoms causing no or minimal interference with usual social and functional activities with intervention not indicated.
- Grade 2 (Moderate): Moderate symptoms causing greater than minimal interference with usual social and functional activities with intervention indicated.
- Grade 3 (Severe): Severe symptoms causing inability to perform usual social and functional activities with intervention or hospitalization indicated.
- Grade 4 (Potentially life-threatening): Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death.

Assessment of Causality

- The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- The relationship of an event to the study intervention (S-648414 or placebo) in Parts 1 and 2 or S-648414 or dolutegravir in Part 3 will be determined by the investigator or subinvestigator according to the following criteria:
  - Related: An AE which can be reasonably explained as having been caused by the study intervention (S-648414 or placebo) in Parts 1 and 2 or S-648414 or dolutegravir in Part 3. For example, the occurrence of the AE can be explained by any of the following: a pharmacological effect of the study intervention (S-648414 or placebo in Parts 1 and 2 or S-648414 or dolutegravir in Part 3 (eg, a similar event had been reported previously); an increase/decrease of the dose affects the occurrence or seriousness of the AE; or all other causative factors (eg, medical history, concomitant medication, etc) can be ruled out after careful analysis of sufficient information.
  - Not related: An AE which cannot be reasonably explained as having been caused by the study intervention (S-648414 or placebo) in Parts 1 and 2 or S-648414 or dolutegravir in Part 3.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The investigator will also consult the Investigator's Brochure and/or Product Information, for marketed products, in his/her assessment.

- The investigator should provide rationale for the causality assessment in the Medical Comment field in EDC or if reporting via paper, the rationale for causality should be provided in the narrative section of paper SAE form.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to CRO/sponsor However, **it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to CRO/sponsor.**
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is 1 of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a study participant dies during participation in the study or during a recognized Follow-up period, the investigator will provide the CRO/sponsor a copy of any postmortem findings including histopathology.
- New or updated information will be recorded in the originally completed eCRF.
- The investigator or qualified designee will submit any updated SAE information to the sponsor within 24 hours of awareness.

Reporting of SAEs

All SAEs must be reported to the CRO/sponsor in detail via EDC or SAE form (if EDC is not available) within 24 hours from the time point when the investigator or qualified designee first becomes aware of the SAE.

SAE Reporting to CRO/Sponsor via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to CRO/sponsor will be the electronic data collection tool.
- If the electronic system (EDC) is unavailable, then the site will use the paper SAE data collection tool (see next section) and enter the collected data into the electronic system when it becomes available. Data collected using the paper SAE data collection tool will be sent to sponsor as follows:

  [redacted]
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the medical monitor/SAE coordinator by telephone.

SAE Reporting to Shionogi via Paper SAE Form (if EDC is unavailable)
• Facsimile transmission of the SAE paper form is the preferred method to transmit this information to the medical monitor. • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE paper form sent by overnight mail or courier service. • Initial notification via telephone does not replace the need for the investigator (or qualified designee) to complete and sign the SAE paper form within the designated reporting time frames.

10.4 Appendix 4: Contraceptive Guidance and Collection of Pregnancy Information

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

Women in the following categories are not considered WOCBP

1. Premenarchal
2. Premenopausal female with 1 of the following:
   - Documented hysterectomy
   - Documented tubal ligation
   - 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. Postmenopausal female
   - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high FSH level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement will be insufficient.
   - Females on HRT and whose menopausal status is in doubt will be required to use one of the nonestrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.
4. Female with amenorrhea due to other medical reasons
   - Women who are in amenorrhea due to other medical reasons such as cancer chemotherapy or drug therapy inducing amenorrhea.

Contraception Guidance:

Male study participants

Male study participants with female partners of childbearing potential are eligible to participate if they agree to ONE of the following during the treatment period and for at least 3 months after the last dose of study intervention:

- Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long-term and persistent basis) and agree to remain abstinent
- Agree to use a male condom plus partner use of a contraceptive method with a failure rate of < 1% per year as described in Table 10-2 when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant

In addition, male participants must refrain from donating sperm for the duration of the study and for 3 months after the last dose of study intervention.

Male participants with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration during the treatment period.

Table 10-2 Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Dependent[a] Failure rate of < 1% per year when used consistently and correctly.
• Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[b] • Oral • Intravaginal • Transdermal
• Progestogen only hormonal contraception associated with inhibition of ovulation • Oral • Injectable
Highly Effective Methods That Are User Independent[a] Failure rate of < 1% per year when used consistently and correctly.
Implantable progestogen only hormonal contraception associated with inhibition of ovulation[b] • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS) • Bilateral tubal occlusion
• **Sexual abstinence** *Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.*

a Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for study participants participating in clinical studies.

b Hormonal contraception may be susceptible to interaction with the study intervention, which may reduce the efficacy of the contraceptive method. In this case, 2 highly effective methods of contraception should be utilized during the treatment period and for at least 3 months after the last dose of study intervention.

Collection of Pregnancy Information:

Details of this procedure are described in Section 8.3.5.

Male participants with partners who become pregnant

- The investigator (or qualified designee) will attempt to collect pregnancy information on any male participant's female partner who becomes pregnant while the male participant is in this study. This applies only to male participants who receive study intervention.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator (or qualified designee) will record pregnancy information on the Pregnancy Form and submit it to the sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female Participants who become pregnant

- The investigator (or qualified designee) will collect pregnancy information on any female participant who becomes pregnant while participating in this study. Information will be recorded on the Pregnancy Form and submitted to the sponsor within 24 hours of learning of a participant's pregnancy. The participant will be followed to determine the outcome of the pregnancy. The investigator (or qualified designee) will collect follow-up information on the participant and the neonate and the information will be forwarded to the sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such. Any poststudy pregnancy-related SAE considered reasonably related to the study intervention by the investigator will be reported to the sponsor as described in Section 8.3.4. While the investigator is not obligated to actively

seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

- Any female participant who becomes pregnant while participating in the study will discontinue study intervention or be withdrawn from the study.

10.5 Appendix 5: Genetics

PGx Research Objective

The objective of the PGx research is to investigate a possible genetic relationship to handling or response to S-648414, or to advance understanding of disease biology (see Section 8.7).

Informed Consent

Participants in the PGx research must voluntarily sign a PGx ICF prior to the blood sample collection for PGx research, in addition to the informed consent for participating in the study body. Study participants who do not wish to participate in the PGx research may still participate in the clinical study.

Management of Sample

For the volume of blood sample to be collected, the time of sample collection, and the storage and shipping condition, see Section 8.7.

The blood sample will be given a specific number to link the sample to study participant and protect personal information (ie, the sample will not be identified with participant personal information by de-identification). Direct personal identifiers (eg, name, address, social security number) will not be transferred to DNA banking facilities and PGx analysis sites.

The sponsor will store extracted DNA samples in the DNA banking facility (BML, INC. in Japan) for up to 10 years after the study completion.

Detailed procedures for sample collection, handling, labeling, storage, and shipping will be specified in a separate document.

PGx Analysis

A separate study protocol will be created to specify the details of the study plan and the site of the PGx analysis. The PGx research will be conducted after obtaining the approval from the ethics committee based on the committee's review about the study's ethics and scientific adequacy. Results for the PGx analysis will be reported in separate documents from the main study results.

All results obtained from the samples will be kept confidential and, if published, all data will be displayed without any personal identifiers. The results of the PGx analysis will not be disclosed to the study participant to avoid disadvantage for the study participant or

his/her family due to misinterpretation of data which may be generated without assay validation to support clinical use.

Study participants can request destruction of his/her blood sample and DNA whenever (even after the clinical study completes); however, the analyzed data including data from the study participant can still be evaluated after consent is withdrawn if PGx analysis has already been completed at the time of withdrawal of consent for PGx analysis.

Study Participant Withdrawal

As stated above, if study participants want to destroy his/her blood sample and DNA, the study participant can request it at any time (even after the clinical study completes).

In addition, a study participant who has consented to participate in PGx research and has a sample collected for PGx research can withdraw from the clinical study for any reason. Regardless whether study participants withdrew the clinical study or not, their blood sample (DNA after extraction) is stored for the PGx research and is destroyed according to procedures on the destruction stated in the previous section.

10.6 Appendix 6: Liver Safety: Suggested Actions and Follow-up Assessments

Phase 1 liver chemistry stopping criteria are designed to assure participant safety and to evaluate liver event etiology.

Phase 1 Liver Chemistry Stopping Criteria and Follow-Up Assessments

<table>
<tr><th colspan="2">Liver Chemistry Stopping Criteria</th></tr>
<tr><td>ALT-absolute</td><td>ALT ≥ 3 x ULN
If ALT ≥ 3 x ULN AND bilirubin ≥ 2 x ULN (> 35% direct bilirubin) or INR > 1.5, report as a SAE[a, b]
See additional actions and follow-up assessments below</td></tr>
<tr><th colspan="2">Required Actions and Follow-up Assessments</th></tr>
<tr><th>Actions</th><th>Follow-Up Assessments</th></tr>
<tr><td>• Immediately discontinue study intervention.
• Report the event to the sponsor's medical monitor within 24 hours.
• Complete the Liver Event Form, and complete an SAE data collection tool if the event also met the criteria for an SAE[b].
• Perform liver function follow-up assessments.
• Monitor the participant daily until liver function test abnormalities resolve, stabilize, or return to baseline (see MONITORING).
MONITORING:
If ALT ≥ 3 x ULN AND bilirubin ≥ 2 x ULN or INR > 1.5</td><td>• Viral hepatitis serology[c].
• Obtain INR and recheck with each liver chemistry assessment until the transaminase values show downward trend.
• Obtain blood sample for PK analysis after the most recent dose[d].
• Serum CPK and LDH.
• Fractionate bilirubin, if total bilirubin ≥ 2 x ULN.
• Complete blood count with differential to assess eosinophilia.
• Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, on the AE eCRF.</td></tr>
</table>

• Repeat liver function tests (include ALT, AST, ALP, bilirubin and INR) and perform liver function follow-up assessments within **24 hours.** • Monitor participant twice weekly until liver function test abnormalities resolve, stabilize, or return to baseline. • A specialist or hepatology consultation is recommended. **If ALT ≥ 3 x ULN AND bilirubin < 2 x ULN and INR ≤ 1.5:** • Repeat liver function tests (include ALT, AST, alkaline phosphatase, bilirubin and INR) and perform liver function follow-up assessments within 24 to 72 hours. • Monitor participants weekly until liver function abnormalities resolve, stabilize, or return to baseline.	• Record use of concomitant medications (including acetaminophen, herbal remedies, and other over-the-counter medications) on the concomitant medications eCRF. • Record alcohol use on the liver event alcohol intake eCRF. **If ALT ≥ 3 x ULN AND bilirubin ≥ 2 x ULN or INR > 1.5:** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total IgG or gamma globulins. • Serum acetaminophen adduct HPLC assay (quantifies potential acetaminophen contribution to liver injury in participants with definite or likely acetaminophen use in the preceding week [10]). • Liver imaging (ultrasound, magnetic resonance, or computerized tomography) and/or liver biopsy to evaluate liver disease; complete liver imaging and/or liver biopsy eCRFs.

AE = adverse event; ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; CPK = creatine phosphokinase; eCRF = electronic case report form; HPLC = high-performance liquid chromatography; IgG = immunoglobulin G; IgM = immunoglobulin M; INR = international normalized ratio; LDH = lactate dehydrogenase; PK = pharmacokinetic; SAE = serious adverse event; ULN = upper limit of normal

a. Serum bilirubin fractionation should be performed if testing is available. If serum bilirubin fractionation is not immediately available, discontinue study intervention if ALT ≥ 3 x ULN and bilirubin ≥ 2 x ULN. Additionally, if serum bilirubin fractionation testing is unavailable, record the absence/presence of detectable urinary bilirubin on dipstick which is indicative of direct bilirubin elevations suggesting liver injury.

b. All events of ALT ≥ 3 x ULN and bilirubin ≥ 2 x ULN (> 35% direct bilirubin) or ALT ≥ 3 x ULN and INR > 1.5 may, if INR measured which may indicate severe liver injury (possible "Hy's Law") and must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis). The INR stated threshold value will not apply to participants receiving anticoagulants.

c. Includes Hepatitis A IgM antibody; HBsAg and HBcAb; hepatitis C RNA; cytomegalovirus IgM antibody; Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, heterophile antibody or monospot testing); and hepatitis E IgM antibody.

d. PK sample may not be required for participants known to be receiving placebo or noncomparator interventions. Record the date/time of the PK blood sample draw and the date/time of the last dose of study intervention prior to the PK blood sample draw on the eCRF. If the date or time of the last dose is unclear, provide the participant's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, do not obtain a PK sample. Instructions for sample handling and shipping are in the Study Reference Manual.

10.7 Appendix 7: Abbreviations and Acronyms

AE	adverse event
Aeu_{0-96}	cumulative amount of the drug excreted in urine during a given collection time interval from 0 to 96 hours
$Aeu_{0-\tau}$	amount of drug excreted in urine over the dosing interval τ
ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
AUC_{0-inf}	area under the concentration-time curve extrapolated from time zero to infinity
AUC_{0-last}	area under the concentration-time curve from time zero to the time of the last quantifiable concentration after dosing
$AUC_{0-\tau}$	area under the concentration-time curve over the dosing interval τ
AUC_{extr}	extrapolated percent of the area under the concentration-time curve from time zero to infinity
$AUMC_{0-inf}$	area under the first moment curve extrapolated to infinity
AV	atrioventricular
BID	twice daily
BLQ	below the lower limit of quantification
BMI	body mass index
bpm	beats per minute
C_τ	plasma concentration at the end of the dosing interval τ (24 hours)
CFR	Code of Federal Regulations
CI	confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CL/F	apparent total clearance
CL_R	renal clearance
C_{max}	maximum plasma concentration
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	coronavirus disease 2019
CPK	creatine phosphokinase

CRO	contract research organization
CRU	clinical research unit
CV	coefficient of variation
CYP3A	cytochrome P450 3A
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
Feu	fraction of dose excreted in urine
$Feu_{0-\tau}$	fraction of dose excreted in urine over the dosing interval τ
Feu_{0-96}	fraction of dose excreted in urine from 0 to 96 hours
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GI	gastrointestinal
eGFR	estimated glomerular filtration rate
HED	human equivalent dose
HIPAA	Health Information Portability and Accountability Act
HIV	human immunodeficiency virus
HR	heart rate
HRT	hormonal replacement therapy
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IN	integrase
IND	investigational new drug
INR	international normalized ratio
IRB	Institutional Review Board
LEDGF	IN-lens epithelium-derived growth factor
LDH	lactate dehydrogenase
MAD	multiple ascending dose(ing)
Mean	arithmetic mean
MDZ	midazolam
MRSD	maximum recommended starting dose
MRT	mean residence time

N	number of nonmissing observations
NOAEL	no observed adverse event level
OTC	over-the-counter
oz.	ounces
PGx	pharmacogenomic(s)
PK	pharmacokinetic(s)
[redacted]	[redacted]
PR	measure between beginning of P wave until beginning of QRS complex
QC	quality control
QD	once daily
QRS	combination of the Q, R, and S waves
QT	QT interval (measure between Q and T wave in heart's electrical cycle)
QTc	corrected QT
QTcI	QTc individual
QTcF	Fridericia's corrected QT
QWBA	quantitative whole body autoradiography
R^2	coefficient of determination
RR	interval from the peak of one QRS complex to the next QRS peak
SAD	single ascending dose(ing)
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SOP	standard operating procedure
Study intervention	S-648414 or placebo
SUSAR	suspected unexpected serious adverse reactions
$t_{1/2,z}$	terminal elimination half-life
T_{max}	time to maximum plasma concentration
ULN	upper limit of normal
Vz/F	apparent volume of distribution in the terminal elimination phase
WHO	World Health Organization
WOCBP	woman of childbearing potential
λ_z	terminal elimination rate constant
Δ	change from baseline
$\Delta\Delta$	placebo-corrected change from baseline

10.8 Appendix 8: Protocol Amendment History

Amendment 5 (06 May 2020)

Overall Rationale for the Amendment:

This amendment was considered necessary to allow the study to expand from a single center in the USA to a 2-center, 2-region study (USA and Japan) to enable enrollment of study participants into Part 3 of the study in either center depending on the risk\impact of coronavirus disease 2019 (COVID-19) around the time of enrollment.

In addition, with this amendment the doses in Part 3 of the study were changed. Based on the unblinded results from Parts 1 and 2 of the study, it was determined that the overall safety profile of the doses evaluated to date was acceptable (see Investigator's Brochure Version 2 Addendum 1 for results). An earlier protocol amendment (Amendment 3) was instituted to reduce the doses of S-648414 in Part 3 based on 3 study participants experiencing adverse events leading to study intervention discontinuation in the 50 mg multiple dose group during Part 2. A planned interim lock of Parts 1 and 2 showed that after unblinding, 2 of the study participants experiencing adverse events leading to discontinuation were on placebo (preferred terms iritis and allergic reaction). It was determined that it would be acceptable to increase the dose in Part 3 to doses that had originally been intended to be assessed (see Investigator's Brochure Version 2 Addendum 1 for further results).

The cohorts affected by this change in dose included:

- Group I changed from S-648414 30 mg (with and without dolutegravir) to S-648414 100 mg (with and without dolutegravir)
- Group J changed from S-648414 50 mg (with and without dolutegravir) to S-648414 200 mg (with and without dolutegravir)

A high-level description of the change(s) and brief scientific rationale for specific items are outlined in the following:

Section(s) # and Name(s)	Description of Change	Brief Rationale
Section 1.1 Synopsis, Objective and Endpoints, Table 1-1 and Section 3 Objectives and Endpoints, Table 3-1	Part 3, added primary objective and endpoint: Objective: • To evaluate the safety and tolerability of S-648414 after administration of multiple oral doses of S-648414 in healthy adult study participants Endpoint: • The number and percentage of clinical and laboratory adverse events in study participants exposed to multiple doses of S-648414	Safety and tolerability of S-648414 (100 mg and 200 mg) are a primary objective, along with pharmacokinetics (PK)

Section(s) # and Name(s)	Description of Change	Brief Rationale
Section 1.1 Synopsis, Overall Design and Section 4.1 Overall Design	Changed single center to multicenter (2 centers)	Added a study center in Japan
Section 1.1 Synopsis, Overall Design, Part 3, Table 1-4, Table 1-5, Section 1.4 Overall Design, Table 4-3, Table 6-3, and Section 6.6.3 Part 3 – Coadministration with Dolutegravir	Changed dose Group I from 30 mg to 100 mg Changed dose Group J from 50 mg to 200 mg	In the multiple-ascending dose portion of this study, 3 study participants in the 50 mg blinded group discontinued due to adverse events. Based on these blinded data, Groups I and J were previously amended to lower the dose for Group I to 30 mg and repeat the 50 mg exposure to gain further safety data at this dose in Group J. On subsequent unblinding, 2 of the 3 discontinuations were placebo recipients and so 50 mg was assessed as safe and escalation of the dose could be considered initially to 100 mg, followed by 200 mg if the escalation criteria are met
Section 1.1 Synopsis, Overall Design, Part 3, Section 4.1 Overall Design, and Section 6.6.3 Part 3 – Coadministration with Dolutegravir	Added safety review criteria for Part 3	The investigator and sponsor will evaluate the results of the safety and tolerability of the 100 mg group before proceeding to the 200 mg group
Section 1.1 Synopsis, Inclusion Criteria and Section 5.1 Inclusion Criteria	Modified inclusion criterion 1 to • clarify lower age (≥ 18 years in USA, ≥ 20 years in Japan) • only include White or Black or African American study participants in Japan	• Clarified lower age limit in Japan based on difference in adult definitions between USA and Japan • Restricted race in Japan to increase global consistency and comparability across results
Section 2.2 Background	Added “The study will also assess the safety and tolerability of multiple doses of S-648414 (100 mg and 200 mg) and the effect of these doses of S-648414 on the PK of dolutegravir and the effect of dolutegravir (50 mg) on the PK of S-648414.”	Clarified new dose groups for multiple doses of S-648414 during Part 3
Section 4.3 Justification for Dose	Changed initial dose in Part 3 from 30 mg/day to 100 mg/day and the subsequent dose from 50 mg/day to 200 mg/day	In the multiple-ascending dose portion of this study, 3 study participants in the 50 mg blinded group discontinued due to adverse events. Based on these blinded data, Groups I and J were previously

Section(s) # and Name(s)	Description of Change	Brief Rationale
		amended to lower the dose for Group I to 30 mg and repeat the 50 mg exposure to gain further safety data at this dose in Group J. On subsequent unblinding, 2 of the 3 discontinuations were placebo recipients and so 50 mg was assessed as acceptably safe and escalation of the dose could be considered initially to 100 mg, followed by 200 mg if the escalation criteria are met
Section 6.1 Study Intervention(s) Administered, Table 6-1 and Table 6-2	Added 200 mg	Added 200 mg group based on change in Part 3
Section 8.2.4 Clinical Safety Laboratory Assessments	For safety laboratory tests, deleted reference to [redacted] laboratory standard operating procedures (SOPs) and revised to local laboratory SOP	With the addition of the study center in Japan, local laboratory of each study site will be used for safety laboratory assessments; therefore, the local laboratory SOPs will be applied
Section 8.7 Genetics	As indicated in the Schedule of Activities, pharmacogenetic blood samples are collected on Day -1 in Part 3	Clarification
Section 10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting	Under "Definition of SAE", deleted examples "eg, hospitalizations for signs/symptoms of the disease under study, death due to progression of disease"	Deleted examples because they are not applicable to healthy study participant population
	Under "Assessment of Causality," clarified that relationship causality is for study intervention (S-648414 or placebo) in Parts 1 and 2 and S-648414 or dolutegravir in Part 3	Clarification

Amendment 4 (16 Mar 2020)

Overall Rationale for the Amendment:

Ocular adverse events were reported during Part 2 of the study and ophthalmological examinations were considered appropriate to be added to Part 3 of the study.

A high-level description of the change(s) and brief scientific rationale for specific items are outlined in the following:

Section(s) # and Name(s)	Description of Change	Brief Rationale
Figure 1-4 and Figure 1-5, Section 8.2.5 Ophthalmological Examinations	Added ophthalmological examinations on Admission Day (Day -1) and at Discharge (Day 29) of Part 3	Added ophthalmological examinations to Part 3 based on recent ocular adverse events reported during Part 2
Figure 1-4	In footnotes d and f, deleted "and Follow-up visit" because this is not applicable to Period 1 of Part 3	Minor editorial revision
Section 4.3 Justification for Dose	Added description of Part 3 including administration with dolutegravir and planned starting and subsequent doses	Added justification for dose in Part 3
Section 5.3.1 Meals and Dietary Restrictions	Clarified that study intervention in Part 3 (ie, Steps 9 and 10) should be administered 30 minutes after initiation of breakfast and no food should be allowed for 4 hours postdose	Clarification
Table 6-1 Study Intervention(s)	Clarified that 240 mL of water should be administered with dolutegravir	Clarification
8.2.1 Physical Examinations, Figure 1-4, and Figure 1-5	Clarified that symptom-focused physical examinations should be conducted for new or worsening adverse events/symptoms.	Clarification

Amendment 3 (09 Feb 2020)

Overall Rationale for the Amendment:

As per Section 6.6.2 of the protocol, upon review of preliminary safety information from multiple-ascending dose (MAD) Group G (50 mg daily), the dose for subsequent MAD cohort (Group H) was adjusted to 30 mg daily with protocol amendment 3. Specifically, although the dose-escalation stopping criteria were not met (as described in Section 6.6.2 of the protocol), there were 3 out of 10 (30%) study participants who received multiple doses of 50 mg (blinded study intervention) and had grade 2 treatment-related adverse events that led to discontinuation of study intervention. Subsequently, the dose was also decreased for the cohort examining the potential for a drug-drug interaction with dolutegravir.

The cohorts affected by this change in dose included:

- Group H changed from S-648414 200 mg to S-648414 30 mg
- Group I changed from S-648414 200 mg (with and without dolutegravir) to S-648414 30 mg (with and without dolutegravir)

A high-level description of the change(s) and a brief scientific rationale for specific items is outlined in the following:

Section(s) # and Name(s)	Description of Change	Brief Rationale
Synopsis, Overall Design Part 2 and Table 1-5, Section 2.2 Background, Section 4.1 Overall Design, Table 4-2, Section 4.3 Justification for Dose, Table 6-3, Section 6.6.2 Part 2- Multiple Dosing, Section 6.6.3 Part 3 – Coadministration with Dolutegravir	Changed dose Group H from 200 mg to 30 mg. Changed dose Group I from 200 mg to 30 mg.	Treatment-related adverse events were observed with multiple doses of 50 mg (Group G) in 30% of subjects; subsequent cohorts with multiple doses were decreased to 30 mg (Groups H and I).
Synopsis, Overall Design, Part 2, Table 1-4, Table 1-5, Section 4.1 Overall Design, Table 4-3, Table 6-3, Section 6.6.3 Part 3 – Coadministration with Dolutegravir, Section 9.5 Interim Analysis	Deleted text pertaining to interim review of Group I.	No interim review is needed now that Group I changed from 200 mg to 30 mg. Interaction with dolutegravir in Part 3 will be assessed with 2 planned doses (30 mg in Group I and 50 mg in Group J).
Synopsis, Study Intervention Groups and Duration	Deleted 100 mg tablets from Parts 2 and 3.	Based on dose change from 200 mg to 30 mg, only 10 mg tablets will be used in Part 2 (Groups G and H) and Part 3 (Groups I and J).
8.3.3 Follow-up of Adverse Events and Serious Adverse Events	Added that all adverse events (AEs) will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up. Previously it	Treatment-related adverse events were observed in Group G (multiple doses of 50 mg). Based on Group G data, all AEs will be followed until resolution, stabilization or until the event is otherwise explained to monitor study safety.

Section(s) # and Name(s)	Description of Change	Brief Rationale
	was only serious adverse events (SAEs) and non-serious AEs of special interest.	
Figure 1-3 and Section 8.7 Genetics	Clarified collection of pharmacogenomics sample should be collected on Admission Day, which is Day -1 in Part 1 and Day -3 in Part 2.	Clarification
Figure 1-3	In footnote d, clarified that vital signs should be collected on Admission Day (Day -3).	Clarification

Amendment 2 (27 Jan 2020)

Overall Rationale for the Amendment:

The primary reason for protocol amendment 2 was to add a third part (Part 3) to the study, where the effect of S-648414 on the PK) of dolutegravir and the effect of dolutegravir on the PK of S-648414 will be studied.

A high-level description of the change(s) and a brief scientific rationale for specific items is outlined in the following:

Section(s) # and Name	Description of Change	Brief Rationale
Title Page	Added "; and A Phase 1 Open-Label Study to Assess the Effect of S-648414 on the Pharmacokinetics of Dolutegravir and the Effect of Dolutegravir on the Pharmacokinetics of S-648414 in Healthy Adult Study Participants (Part 3)"	To add Part 3
	Updated to Amendment Number 2.	Minor editorial revision
1.1. Synopsis, Protocol Title	Added "; and A Phase 1 Open-Label Study to Assess the Effect of S-648414 on the Pharmacokinetics of Dolutegravir and the Effect of Dolutegravir on the Pharmacokinetics of S-648414 in Healthy Adult Study Participants (Part 3)"	To add Part 3
1.1 Synopsis, Rationale	Added the effect of S-648414 on the PK of dolutegravir and the effect of dolutegravir on the PK of S-648414.	To add Part 3
1.1 Synopsis, Objectives and Endpoints, Table 1-1 Objectives and Endpoints and 3 Objectives and Endpoints, Table 3-1 Objectives and Endpoints	Added primary objectives: to evaluate the effect of S-648414 on the PK of dolutegravir and the effect of dolutegravir on the PK of S-648414 and to add primary endpoints: C_{max}, T_{max}, C_{τ}, AUC, and CL/F in Part 3.	Added primary and secondary objectives and endpoints for Part 3

Section(s) # and Name	Description of Change	Brief Rationale
	Added secondary objective: to evaluate the safety and tolerability of S-648414 coadminstered with dolutegravir and to add secondary endpoints of number and percentage of clinical and laboratory adverse events in Part 3.	
	Added definition for C_τ in the footnotes.	Minor editorial revision
1.1 Synopsis, Overall Design	Added the effect of S-648414 on the PK of dolutegravir and the effect of dolutegravir on the PK of S-648414.	To add Part 3
	Updated table and figure cross reference numbering (changed Table 1-3 to Table 1-5, changed Figure 1-1 to Figure 1-2, and Figure 1-2 to Figure 1-3), added "of" in header of Table 1-2 and Table 1-3.	Minor editorial revisions
	Added study design details for Part 3 including open-label, non-randomized, 1-sequence, 3-period study. Added study period and treatment details for Part 3. Added Table 1-4 Part 3 Treatment Schedule.	To add study design details for Part 3
1.1 Synopsis, Inclusion Criteria and 5.1 Inclusion Criteria	Criterion 4, clarified administration of "study intervention".	Minor editorial revision
	Criterion 4, added details that clinical evaluations can be repeated (for confirmatory purposes or if additional information is required to assess study participant's eligibility) at the discretion of the investigator; repeated procedures must be performed within the same Screening window.	To add details to allow for repeat evaluations during Screening
1.1 Synopsis, Exclusion Criteria and 5.2 Exclusion Criteria	Criterion 23, clarified amount of blood donated as > 500 mL.	Minor editorial revision
1.1 Synopsis, Number of Study Participants and 9.2 Sample Size Determination	Added "28 healthy adult study participants in Part 3"	To add number of study participants for Part 3
1.1 Synopsis, Study Intervention, Groups and Duration	Added "S-648414 tablets 10 mg or 100 mg in the fed state (Groups I and J)"	To add dose strength in Part 3
1.1 Synopsis, Other Treatment, Dose, and Mode of Administration	Added "Dolutegravir 50-mg tablet, oral administration in the fed state"	To add other treatment required during Part 3
1.1 Synopsis, Duration of Study Intervention	Added "Multiple doses of dolutegravir once daily during Period 1, multiple doses of S-648414 once daily during Period 2, and multiple doses of S-648414 coadministered with dolutegravir once daily during Period 3."	To add study intervention during Part 3

Section(s) # and Name	Description of Change	Brief Rationale
1.1 Synopsis, Study Duration for Individual Study Participants (Includes Screening, Treatment and Follow-up Period)	Added 5 to 9 weeks for Part 3.	To add duration during Part 3
1.1 Synopsis, Pharmacokinetic Assessments	Added PK assessments (C_{max}, T_{max}, C_{τ}, $AUC_{0-\tau}$, and CL/F) for Part 3.	To add PK assessments for Part 3
1.1 Synopsis, Safety Assessments	Clarified that ophthalmological examinations are collected in Parts 1 and 2.	Ophthalmological examinations not collected in Part 3
1.1 Synopsis, Statistical Methods	Added details for PK analysis (ie, ANOVA model) for Part 3.	To add PK statistical methods for Part 3
	Clarified that ophthalmological examinations are collected in Parts 1 and 2.	Ophthalmological examinations not collected in Part 3
1.1 Synopsis, Table 1-5	Updated table numbering from Table 1-4 to Table 1-5.	Minor editorial revisions
	Added rows and footnotes for Groups I and J.	To add Part 3
1.2 Schema	Added "Figure 1-1 Study Schematic - Part 3" and updated figure cross referencing (updated Figure 1-1 to Figure 1-2, Figure 1-2 to Figure 1-3) and added cross referencing for Part 3 (Figure 1-4 and Figure 1-5).	To add figure and minor editorial changes
1.3 Schedule of Activities Part 1, Figure 1-2	Updated figure numbering from Figure 1-1 to Figure 1-2.	Minor editorial revision.
	Changed collection of PGx sample from predose on Day 1 to Admission Day (Day -1)	For consistency across Parts 1 to 3 of the study
	Footnote h - changed optometrist to ophthalmologist.	Clarified specialist
1.3 Schedule of Activities Part 2, Groups G and H, Figure 1-3	Updated figure numbering from Figure 1-2 to Figure 1-3. Corrected grammar in footnote i.	Minor editorial revision
	Changed collection of PGx sample from predose on Day 1 to Admission Day (Day -1)	For consistency across Parts 1 to 3 of the study
	Deleted (S-648414 or placebo) after intervention.	Minor editorial revision
	In footnote g, changed optometrist to ophthalmologist.	Clarified specialist
1.3 Schedule of Activities, Figure 1-4 and Figure 1-5	Added Figure 1-4 Schedule of Activities – Part 3, Period 1, Groups I and J and Figure 1-5 Schedule of Activities – Part 3, Periods 2 and 3, Groups I and J.	To add schedule of activities for Part 3

Section(s) # and Name	Description of Change	Brief Rationale
2.1 Study Rationale	Added "as well as the effect on PK of dolutegravir and the effect of dolutegravir on PK of S-648414."	To add Part 3
4.1 Overall Design	Updated figure cross reference numbering (changed Figure 1-1 to Figure 1-2, and Figure 1-2 to Figure 1-3).	Minor editorial revisions
4.1 Overall Design, Part 3 and Table 4-3	Added study design, treatment details, and Part 3 Treatment Schedule for Part 3.	To add Part 3
4.2 Scientific Rationale for Study Design	Added details for Part 3.	To add Part 3
5.4 Screen Failures	Deleted paragraph regarding screen failures.	Deleted text and revised Inclusion Criterion 4 in Sections 1.1 and 5.1, which allows for repeated assessments
6.1 Study Intervention(s) Administered, Table 6-1 Study Intervention(s)	Clarified unit dose of midazolam is 2 mg/mL and dosage level is 5 mg	Minor editorial revision
	Added "1 bottle per box" for S-648414 and placebo	Clarified that packaging includes 1 bottle per box
	Changed manufacturer for midazolam.	Updated to indicate generic versions are acceptable
	Added study intervention details for dolutegravir.	To add dolutegravir for Part 3
6.1 Study Intervention (s) Administered, Table 6-2 Number of Tablets (S-648414 or Placebo) per Dose Group	Added or "(S-648414 or Placebo)" to title.	Minor editorial revision
6.2 Preparation/Handling/ Storage/Accountability of Study Intervention	Item 1, deleted "and protected from light."	Study intervention does not need to be protected from light.
6.3 Measures to Minimize Bias: Randomization and Blinding; 7.1 Discontinuation of Study Intervention; 7.2 Study Participant Discontinuation/ Withdrawal from the Study; 8 Study Assessments and Procedures; 8.2 Safety Assessments; 8.2.4 Clinical Safety Laboratory Assessments; 8.2.5 Ophthalmological Examinations	Updated figure cross reference numbering (changed Figure 1-1 to Figure 1-2, and Figure 1-2 to Figure 1-3), and added cross-references for Figure 1-4 and Figure 1-5.	Minor editorial revisions

Section(s) # and Name	Description of Change	Brief Rationale
6.6 Dose Modification of Study Intervention, Table 6-3 Sequence of Steps	Added rows and footnotes for Groups I and J.	To add Part 3
6.6.3 Part 3 – Coadministration with Dolutegravir	Added treatment details for Groups I and J.	To add Part 3
7.1 Discontinuation of Study Intervention	Clarified that this text pertaining to discontinuation of study intervention applies to Part 2 and Part 3	To add Part 3
8.2.3 Electrocardiograms	Changed to clarify only Holter equipment will be supplied by ERT.	Minor clarification
8.2.3.2 Expert-Precision QT Analysis	In second paragraph, changed “cardiologist” to “cardiac safety specialist”.	Minor clarification
8.2.5 Ophthalmological Examinations	Changed optometrist to ophthalmologist.	Clarified specialist
8.3.1 Time Period and Frequency for Collecting Adverse Events and Serious Adverse Event Information, 8.3.3 Follow-up of Adverse Events and Serious Adverse Events, 8.3.4 Regulatory Reporting Requirements for Serious Adverse Events, 8.4 Special Situations – Abuse, Misuse, Overdose, and Medication Error, 10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting	Modified wording to indicate that SAEs will be recorded “in the eCRF” and that the investigator or “qualified designee” can submit AE/SAE initial or follow-up information.	Clarified that AE/SAE reports and updates can be submitted by the investigator or qualified designee.
8.3.1 Time Period and Frequency for Collecting Adverse Events and Serious Adverse Event Information	Added paragraph “Investigators or qualified designees are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the investigator or qualified designee learns of any SAE, including a death, at any time after a participant is no longer in the study, and the investigator or qualified designee considers the event to be reasonably related to the study treatment or study participation, the investigator or qualified designee must promptly notify the sponsor via phone, e-mail, or fax (see Section 10.3 [Appendix 3]). Investigator assessment of causality must be included with all SAEs reported to the sponsor. Serious adverse events with missing investigator causality	To add instructions for collection of SAEs after conclusion of study participation

Section(s) # and Name	Description of Change	Brief Rationale
	will be followed up by the CRO urgently until response is provided to the sponsor."	
8.5 Pharmacokinetics	Updated figure cross reference numbering (changed Figure 1-1 to Figure 1-2, and Figure 1-2 to Figure 1-3).	Minor editorial revisions
	Added amount of plasma required for Part 3.	To add Part 3
	Clarified that samples are to be shipped to bioanalytical laboratory at [redacted]	Minor clarification
8.7 Genetics	Second paragraph, changed collection of blood sample from Day 1 to Day -1.	Changed to match schedule of assessments
8.8 Biomarkers	Updated figure cross reference numbering (changed Figure 1-2 to Figure 1-3), corrected maximum storage requirements to -85ºC. Added shipping address for biomarkers.	Minor editorial revisions
9.1 Statistical Hypotheses	Added effect on PK of dolutegravir and effect of dolutegravir on PK of S-648414.	To add Part 3
9.3 Populations for Analyses	Added dolutegravir to definition of PK concentration population.	To add Part 3
9.4 Statistical Analyses	For the SAP, deleted "have a version 1.0 available by first patient randomized, and"	Clarified that SAP will finalized before unblinding/database lock but not before first patient randomized.
9.4.3 Safety Analyses, Adverse event	Clarified study intervention definition from S-648414 or placebo to S-648414, midazolam, dolutegravir, or placebo.	To clarify study intervention definition for AEs leading to study intervention withdrawal
9.4.4.1 Pharmacokinetic Analyses	Added primary endpoints of effect of S-648414 on PK of dolutegravir and effect of dolutegravir on PK of S-648414.	To add Part 3
9.4.4.1.1 Plasma and Urine Concentration	Added "and dolutegravir" and "and in Part 3 achievement of steady state for plasma S-648414 and dolutegravir."	To add Part 3
9.4.4.1.2 Pharmacokinetic Parameters	Added "and dolutegravir" Added PK parameters and definitions for Part 3.	To add Part 3
9.4.4.1.8 Effect of S-648414 on the Pharmacokinetics of Dolutegravir	Added statistical analysis details for the effect of S-648414 on the PK of dolutegravir.	To add Part 3
9.4.4.1.9 Effect of Dolutegravir on the Pharmacokinetics of S-648414	Added statistical analysis details for the effect of dolutegravir on the PK of S-648414.	To add Part 3

Section(s) # and Name	Description of Change	Brief Rationale
9.5 Interim Analyses	Added "To assist with the evaluation and dose selection for the S-648414 Proof of Concept study, the sponsor will be unblinded after the completion of Part 2 and an evaluation of safety and PK data from Parts 1 and 2 will be conducted.	To add unblinded analyses by the sponsor after completion of Part 2
	Added "For open-label Part 3, Group J will receive 50 mg of S-648414 and will only be conducted if relevant interaction is observed with dolutegravir and S-648414 based on interim review of Group I (see Section 6.6.3 for Part 3)."	To add Part 3
10.2 Appendix 2: Clinical Laboratory Tests	Changed "central" to "local" and deleted second bullet because not relevant as not using a central laboratory.	Clarification that local laboratory is being used during the study and not a central laboratory
10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting	Modified last bullet in Follow-up of AEs and SAEs information to indicate that SAE information is due to the sponsor within 24 hours of awareness. Clarified that SAE information is to be submitted by the investigator "or qualified designee" Changed "Shionogi" to "sponsor" for the SAE data collection.	Clarified that SAE updates can be submitted by the investigator or qualified designee and that SAE information is to be submitted within 24 hours of awareness
	Added section for "SAE Reporting to Shionogi via Paper SAE Form (if EDC is unavailable)" Facsimile transmission of the SAE paper form is the preferred method to transmit this information to the medical monitor. In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE paper form sent by overnight mail or courier service. Initial notification via telephone does not replace the need for the investigator (or qualified designee) to complete and sign the SAE paper form within the designated reporting time frames.	Added details for reporting SAEs to Shionogi via paper form if EDC is unavailable
10.4 Appendix 4: Contraceptive Guidance and Collection of Pregnancy Information	Clarified that collection and reporting of pregnancy information can be made by investigator or "qualified designee"	Clarification that investigator or qualified designee can collect and report pregnancy information
10.6 Appendix 6: Liver Safety: Suggested Actions and Follow-up Assessments	Clarified that events should be reported to the sponsor "medical monitor"	Minor clarification

Section(s) # and Name	Description of Change	Brief Rationale
10.7 Appendix 7: Abbreviations and Acronyms	Added C_τ and corresponding definition.	Added based on Part 3
10.8 Appendix 8: Protocol Amendment History	Added rationale and list of changes for Amendment 1	Added protocol amendment history for Amendment 1

Amendment 1 (23 Dec 2019)

Overall Rationale for the Amendment:

The primary reason for protocol amendment 1 was to modify the dose in Groups G and H in Part 2 based on preliminary data from Groups A, B, C, and D of this study.

Human pharmacokinetic data from single ascending dose (SAD) cohorts 10 mg through 250 mg demonstrated higher exposures than predicted and no safety concerns were identified.

Based on these data, proposed doses of 100 mg and 300 mg for the multiple ascending dose (MAD) cohorts were reduced to 50 mg and 200 mg. The 50 mg and 200 mg doses are predicted to achieve sufficient exposures for anti-viral effect.

A high-level description of the change(s) and a brief scientific rationale for specific items is outlined in the following:

Section(s) # and Name(s)	Description of Change	Brief Rationale
Synopsis, Overall Design Part 2, Table 1-3, and Table 1-4 Section 4.1 Overall Design, Table 4-2, Section 4.3, Table 6-1, Table 6-2, Table 6-3, Section 6.6.2	Changed dose Group G from 100 mg to 50 mg Changed dose Group H from 300 mg to 200 mg	Although no safety concerns were identified, doses were decreased based on higher exposures than predicted in Groups A, B, C, and D of this study.
Synopsis, Test Drug, Dose, and Mode of Administration	Part 2 – changed 100 mg tablets in Groups G and H to 10 mg or 100 mg tablets	10 mg tablet added since Group G was changed from 100 mg to 50 mg.

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Final Approval	[redacted]

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