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	Statistical Analysis Plan Version 4 10 Aug 2020

History of Statistical Analysis Plan Amendments

Version 1 (Original)	22 Nov 2019
Version 2	3 Apr 2020
Version 3	10 Apr 2020
Version 4	10 Aug 2020
Revisions to Version 1, 2, 3 and 4 are documented in Version 4	

Shionogi, Inc.

1908T0911

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)

10AUG2020

Final Statistical Analysis Plan Amendment 3

Version 4.0

Prepared by:

[redacted]

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Document History

Version	Date	Changes
1.0	22NOV2019	Original version
2.0	03APR2020	1) Protocol Version change from Version original dated 30 July 2019 to Version Amendment 3 dated 09 Feb 2020. 2) Part 3 was added. 3) Part 2 dose were updated from Group G 100mg and Group H 300 mg to Group G 50 mg and Group H 30 mg. 4) Section 4.3: Analysis Populations were updated. 5) Section 9.3: the summary method for 3 consecutive readings for blood pressure was added 6) Pharmacogenomics Sample Collection were deleted from Section 9.8
3.0	10APR2020	1) Section 9.1: Additional Ocular Adverse Events of Special Interest were added
4.0	10AUG2020	1) Section 2.3, Part 3 Objectives updated per Protocol Amendment 6 2) Section 3.1.3 Part 3 Overall Study Design, Days 9 to 13, changed from outpatient to either inpatient or outpatient per Protocol Amendment 6 3) The dose for Groups I and J changed from 30 mg and 50 mg to 100 mg and 200 mg per Protocol Amendment 6

List of Abbreviations

AE	adverse event
Aeu_{x-y}	amount of the drug excreted in urine during a given collection time interval from x to y 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
BID	twice daily
BLQ	below the lower limit of quantification
BMI	body mass index
Cτ	Plasma concentration at the end of the dosing interval τ (24 hours) on Day 7 and Day 28
CI	confidence interval
CL/F	apparent total clearance
CL_R	renal clearance
C_{max}	maximum plasma concentration
CPK	creatine phosphokinase
CRU	clinical research unit
CSR	clinical study report
Cτ	plasma concentration at the end of the dosing interval τ (24 hours)
CV	coefficient of variation
CYP3A	cytochrome P450 3A
ECG	electrocardiogram
eCRF	electronic case report form
Feu	fraction of dose excreted in urine
$Feu_{0-\tau}$	fraction of dose excreted in urine over the dosing interval τ
Feu_{x-y}	fraction of dose excreted in urine from x to y hours
FSH	follicle-stimulating hormone
GI	gastrointestinal
HIV	human immunodeficiency virus
IEC	Independent Ethics Committee
INR	international normalized ratio
IRB	Institutional Review Board
LDH	lactate dehydrogenase
MAD	multiple ascending dose(ing)
MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
MDZ	midazolam

MedDRA	Medical Dictionary for Regulatory Activities
MRT	mean residence time
PGx	pharmacogenomic(s)
PK	pharmacokinetic(s)
[redacted]	[redacted]
PT	preferred term
PR	measure between beginning of P wave until beginning of QRS complex
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
R^2	coefficient of determination
RBC	red blood cell
SAD	single ascending dose(ing)
SAE	serious adverse event
SD	standard deviation
SOC	system organ class
$t_{1/2,z}$	terminal elimination half-life
TEAE	treatment-emergent adverse event
T_{max}	time to maximum plasma concentration
ULN	upper limit of normal
V_z/F	apparent volume of distribution in the terminal elimination phase
WBC	white blood cell
WHO	World Health Organization
λz	terminal elimination rate constant
Δ	change from baseline
$\Delta\Delta$	placebo-corrected change from baseline

1. Introduction

This document outlines the statistical methods to be implemented during the analysis of data collected within the scope of Shionogi, Inc., protocol 1908T0911 (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 (MDZ), in Healthy Adult Study Participants [Part 2]; and A Phase 1 Open-Label Study to Assess the Effect of S-648414 on the Pharmacokinetics (PK) of Dolutegravir and the Effect of Dolutegravir on the Pharmacokinetics of S-648414 in Healthy Adult Study Participants [Part 3]), Amendment 6, dated 17 Jun 2020. The purpose of this statistical analysis plan is to define the planned statistical analysis of the study data consistent with the study objectives.

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 compliant toxicology studies. These Phase 1 assessments will describe the safety, tolerability, and 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 MDZ, 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.

2. Objectives

2.1. Part 1 Objectives

The primary objective is

- To evaluate the safety and tolerability of S-648414 after administration of a single oral dose of S-648414 in healthy adult study participants.

The secondary objectives are

- 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

2.2. Part 2 Objectives

The primary objective is

- To evaluate the safety and tolerability of S-648414 after administration of multiple oral doses of S-648414 in healthy adult study participants

The secondary objectives are

- 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

2.3. Part 3 Objectives

The primary objective is

- 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 S648414 in healthy adult study participants
- To evaluate the safety and tolerability of S-648414 after administration of multiple oral doses of S-648414 in healthy adult study participants.

The secondary objectives are

- To evaluate the safety and tolerability after coadministration of dolutegravir with S-648414 in healthy adult study participants

3. Investigational Plan

3.1. Overall Study Design and Plan

The study is a 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 12.2.

3.1.1. Part 1 Overall Study Design and Plan

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).

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 3.1.1.1).

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 3.1.1.1 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 [serious adverse event] 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 events (AEs)
- ≥ 25% of study participants experience grade 3 or 4 treatment-related AEs

See Protocol 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.

The overall schedule of events for Part 1 is provided in Table 12.1.1.

3.1.2. Part 2 Overall Study Design and Plan

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 3.1.2.1). 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 MDZ 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 Table 12.1.2).

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 3.1.2.1 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 Protocol 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.

The overall schedule of events for Part 2 is provided in Table 12.1.2.

3.1.3. Part 3 Overall Study Design and Plan

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 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 3.1.3.1 for the study schematic.

Table 3.1.3.1 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 (of which 5 days are out patient) 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 overall schedule of events for Part 3 is provided in Table 12.1.3.1 and Table 12.1.3.2.

3.2. Study Endpoints

3.2.1. Part 1 Study Endpoints

The primary endpoint for Part 1 is:

- The number and percentage of clinical and laboratory AEs in study participants exposed to single dose of S-648414

The secondary endpoints for Part 1 are:

- S-648414: C_{max}, T_{max}, AUC, $t_{1/2,z}$, λ_z, MRT, CL/F, V_z/F, CLR, and Feu following single oral dosing, 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

3.2.2. Part 2 Study Endpoints

The primary endpoint for Part 2 is:

- The number and percentage of clinical and laboratory AEs in study participants exposed to multiple doses of S-648414

The secondary endpoints for Part 2 are:

- S-648414: C_{max}, T_{max}, AUC, $t_{1/2,z}$, λ_z, MRT, CL/F, V_z/F, CLR, and Feu
- MDZ: C_{max}, T_{max}, AUC, $t_{1/2}$,z, λ_z, and MRT

3.2.3. Part 3 Study Endpoints

The primary endpoint for Part3 is:

- 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

The secondary endpoint for Part 3 is:

- The number and percentage of clinical and laboratory AEs in study participants exposed to multiple doses of S-648414 and dolutegravir

4. General Statistical Considerations

All statistical analyses will be conducted using statistical analysis system SAS® Version 9.4 or higher (SAS Institute, Cary, NC). For tables and data listings with no data, all titles, footnotes and column headers will be presented, and "No data to display" will be presented. All tables and data listings will appear in landscape format employing Courier New 9-point black font.

Unless otherwise noted, continuous variables will be summarized by using the number of nonmissing observations, arithmetic mean, standard deviation (SD), 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.

Unless otherwise noted, minimum and maximum will be presented to the same number of decimal places as the raw data, mean and median will be presented to one more decimal place than the raw data, and SD will be presented to one more decimal place than the raw data.

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

All data listings will be sorted by part and subject number.

No algorithm for imputation of missing data will be employed.

For summary of safety assessments, if there are repeated measurements at a time point, the original measurement at that time point will be used in the summary tables. If the original measurement is missing, the next available measurement at that time point will be used in the summary tables.

For Part 1 (other than Group C) and Part 2, baseline will be defined as the last non-missing assessment (including repeated and unscheduled assessments) before the first dose of any study interventions (S-648414, Midazolam, or Placebo), unless otherwise specified. For Part 1 Group C, baseline will be defined as the last non-missing assessment (including repeated and unscheduled assessments) before the first dose of any study interventions (S-648414, Midazolam, or Placebo) for each Period (C1 and C2), unless otherwise specified. For Part 3, Baseline will be defined as the last non-missing assessment (including repeated and unscheduled assessments) before the first dose of any study interventions (Dolutegravir), unless otherwise specified. Unscheduled results will not be included in the summary tables except for determining baseline but will be presented in data listings.

For Parts 1 and 2, Study Day will be defined as the day relative to the first exposure of either S-648414 or placebo. For Part 3, Study Day will be defined as the day relative to the first exposure of Dolutegravir in Period 1. Previous day to Study Day 1 is defined as Day -1 (there is no Study Day 0).

In Part 1 and Part 2, all subjects receiving placebo will be pooled across groups for the summaries.

4.1. Sample Size

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.

4.2. Randomization, Stratification, and Blinding

For Part 1 and Part 2, [redacted] will generate the randomization schedule. Eligible subjects within each group will be randomly assigned after all entry criteria have been satisfied before the first dose of study intervention administration. Each group will be independently randomly assigned by a qualified person who is not directly involved in study conduct, data management, or data analysis.

Part 1 and Part 2 are double-blind study. Neither the subjects nor the Investigator will be aware of the treatment assignment. Blinding will be maintained throughout the study by use of active and placebo dosage forms of similar appearance. Access to the randomization code will be strictly controlled according to the standard operating procedures of [redacted].

[redacted] will provide the pharmacy with the randomization schedules for the study and with subject-specific blind breaking documents for use in the case of the need for emergency unblinding. 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.

Stratification will not be used in this study.

Part 3 is open-label, non-randomized.

4.3. Analysis Populations

The enrolled population will include all participants who sign the informed consent (ICF) for Part 1 and Part 2, and all participants who sign the ICF and passed screening for Part 3.

The randomized population will include all participants randomly assigned to study intervention for Part 1 and Part 2. Participants will be analyzed according to the intervention they planned to be received.

The safety population will include all participants who are randomly assigned to study intervention, and take at least 1 dose of study intervention for Part 1, Part 2, and all participants who take at least 1 dose of study intervention for Part 3. Participants will be analyzed according to the intervention they actually received.

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 and plotting of the individual concentration-time data.

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 and plotting of PK parameter versus dose data. This population will also be used for plotting of the mean concentration-time data, the concentration summary and statistical analysis.

5. Subject Disposition

5.1. Subject Disposition

The number of screen failures and reasons of screen failures will be summarized. 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. In addition, the number of study participants excluded from each analysis population with those reasons will be summarized.

Subject disposition data, screen failure, analysis populations, and randomization data will be presented in data listings.

5.2. Protocol Deviations

Significant protocol deviations will be summarized by treatment group and overall. Participants could have more than one significant protocol deviation, but are counted once for each level of summarization. Protocol deviations will be presented in a data listing. The details of protocol deviation category will be defined in a separate document.

6. Demographics and Baseline Characteristics

6.1. Demographics

Demographic and baseline characteristics will be summarized by treatment group and overall for each part with descriptive statistics for the safety population. Descriptive statistics will be calculated for the following continuous demographic characteristics: age (years), weight (kg), height (cm), and body mass index (BMI, kg/m^2). Frequency counts will be tabulated for the categorical variables of sex (Female, or Male), race (American Indian or Alaska Native, Asian, Black or African American, White, or Other), and ethnicity (Hispanic or Latino, Not Hispanic or Latino, Not Reported, or Unknown).

Demographic information collected at screening will be summarized and presented in a data listing.

6.2. Medical History

The medical history data will be coded using the Medical Dictionary for Regulatory Activities (MedDRA; version to be delineated in the clinical study report [CSR]) and presented in a data listing.

6.3. Inclusion and Exclusion Criteria

All inclusion/exclusion criteria deviations recorded in the electronic case report form (eCRF) will be presented in a data listing.

7. Treatments and Medications

7.1. Prior and Concomitant Medications

7.1.1. Prior Medications

Prior medications will be defined as medications that stopped before the first dose of study intervention. 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.

7.1.2. Concomitant Medications

Concomitant medications will be defined as medications that started after the date of the first dose of study intervention up to the subject's last visit, or medications that started before the first dose of study intervention and are ongoing after the first dose of study intervention.

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.

7.2. Medical or Surgical Treatment Procedures

Medical or surgical treatment procedures will be presented in a data listing.

7.3. Study Intervention Administration

The study intervention exposure will be presented in a data listing.

7.4. Meals

Meal data will be presented in a data listing.

8. Pharmacokinetic Analysis

8.1. Pharmacokinetic Sample Concentrations

Blood and urine samples will be collected and analyzed for S-648414 in plasma and urine at the following time points:

Part 1 (S-648414):

Sample Type	Scheduled Time (hours)
Plasma	Predose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 24, 48, 72 and 96 hours postdose.
Urine	Predose (-12 to 0 hours), 0 to 24 hours, 24 to 48 hours, 48 to 72 hours, and 72 to 96 hours postdose

Part 2 (S-648414):

Sample Type	Dosing Day	Scheduled Time (hours)
Plasma	1	Predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hours postdose
Plasma	5, 7, 10, 12, 13	Predose
Plasma	14	Predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 24, 48, 72, and 96 hours postdose
Urine	14	0-24 hours postdose

For Part 2, blood samples will be collected and analyzed for MDZ in plasma at the following time points:

Part 2 (MDZ):

Sample Type	Dosing Day	Scheduled Time (hours)
Plasma	-2	Predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hours postdose.
Plasma	14	Predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hours postdose.

For Part 3, blood samples will be collected and analyzed for S-648414 in plasma at the following time points:

Part 3 (S-648414):

Sample Type	Dosing Day	Scheduled Time (hours)
Plasma	15, 18, 19, 20	Predose
Plasma	21	Predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 12 hours postdose.
Plasma	22, 25, 26, 27	Predose
Plasma	28	Predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hours postdose.

For Part 3, blood samples will be collected and analyzed for dolutegravir in plasma at the following time points:

Part 3 (Dolutegravir):

Sample Type	Dosing Day	Scheduled Time (hours)
Plasma	1, 4, 5, 6	Predose
Plasma	7	Predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12 and 24 hours postdose.
Plasma	22, 25, 26, 27	Predose
Plasma	28	Predose (0 hours), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hours postdose.

For each part, plasma concentrations of S-648414, MDZ (Part 2 only) and dolutegravir (Part 3 only) will be listed for each subject and time point and summarized by dosing regimen with number of subjects in a given dose cohort (N), the number of nonmissing observations (n), arithmetic mean (mean), SD, coefficient of variation (CV%, calculated by SD/mean × 100), geometric mean, coefficient of variation for geometric mean (geometric CV%), median, minimum, and maximum values at each nominal 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 for natural log (ln)-transformed data.

For each part, mean plasma concentration versus nominal time profiles will be presented for each dosing regimen on both linear and semi-logarithmic scales. Individual plasma concentration versus actual time profiles will be grouped by dosing regimen and presented on both linear and semi-logarithmic scales.

In Part 2, achievement of steady state for plasma S-648414 concentration will be assessed by visual inspection of trough concentration data.

In Part 3, achievement of steady state for plasma S-648414 and dolutegravir concentrations 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% values.

For each part, urine volume and urine concentration data for S-648414 will be listed by subject and collection interval. In Part 1, time course profiles for cumulative fraction of dose excreted in urine will be presented graphically.

Descriptive statistics will be presented as follows:
- N and n: no decimal place
- Arithmetic mean, geometric mean, SD, median, Min, and Max: same precision as per the observed values
- CV% and Geometric CV%: one decimal place

8.2. Pharmacokinetic Parameters

Pharmacokinetic parameters will be calculated whenever possible for S-648414, MDZ and dolutegravir from plasma concentration data by noncompartmental methods using Phoenix® WinNonlin® Version 8.0 or higher (Certara USA, Inc., Princeton, NJ). Parameters of S- 648414 from urine volume and concentration data will be calculated with SAS Version 9.4 or higher. 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:

PK Parameter	Definition
Plasma	
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

AUC_{extr} (%)	Extrapolated percent of $AUC_{0\text{-}inf}$, calculated as AUC_{extr} (%) = 100 × ($AUC_{0\text{-}inf}$ − $AUC_{0\text{-}last}$)/$AUC_{0\text{-}inf}$
$t_{1/2,z}$ (hr)	Terminal elimination half-life, where $t_{1/2,z} = (\ln 2)/\lambda_z$
λz (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: $V_z/F = Dose/AUC_{0\text{-}inf}/\lambda_z$
Urine	
$Aeu_{x\text{-}y}$ (mg)	Amount of the drug excreted in urine during a given collection time interval from x to y hours ($Aeu_{0\text{-}24}$, $Aeu_{24\text{-}48}$, $Aeu_{48\text{-}72}$, $Aeu_{72\text{-}96}$, $Aeu_{0\text{-}96}$)
$Feu_{x\text{-}y}$ (%)	Fraction of dose excreted in urine from x to y hours; calculated as $Aeu_{x\text{-}y}/Dose \times 100$ ($Feu_{0\text{-}24}$, $Feu_{24\text{-}48}$, $Feu_{48\text{-}72}$, $Feu_{72\text{-}96}$, $Feu_{0\text{-}96}$)
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:

PK Parameter	Definition
Plasma	
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
$AUC_{0-\tau}$ (ng·hr/mL)	Area under the concentration-time curve over the dosing interval 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: $CL/F = Dose/AUC_{0-\tau}$ on Day 14
V_z/F (L)	Apparent volume of distribution in the terminal elimination phase on Day 14, estimated according to: $V_z/F = Dose/AUC_{0-\tau}/\lambda_z$
Urine	
$Aeu_{0-\tau}$ (mg)	Amount of the drug excreted in urine over the dosing interval τ on Day 14
$Feu_{0-\tau}$ (%)	Fraction of dose excreted in urine over the dosing interval τ (24 hours) on Day 14; calculated as $Aeu_{0-\tau}/Dose \times 100$, where $Aeu_{0-\tau}$ is the amount of drug excreted in urine over the dosing interval τ (24 hours)
CL_R (L/hr)	Renal clearance on Day 14, calculated as $CL_R = Aeu_{0-\tau}/AUC_{0-\tau}$, where $Aeu_{0-\tau}$ is the amount of drug excreted in urine over the dosing interval τ (24 hours)

Pharmacokinetic parameters for MDZ on Day -2 and Day 14 for Part 2 include:

PK Parameter	Definition
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 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
AUC_{extr} (%)	Extrapolated percent of $AUC_{0\text{-}inf}$, calculated as AUC_{extr} (%) = 100 × ($AUC_{0\text{-}inf} - AUC_{0\text{-}last}$)/$AUC_{0\text{-}inf}$
$t_{1/2,z}$ (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 for S-648414 in Part 3 on Day 15 and Day 28 include:

PK Parameter	Definition
C_{max} (ng/mL)	Maximum plasma concentration
T_{max} (hr)	Time to maximum plasma concentration
C_τ	Plasma concentration at the end of the dosing interval τ (24 hours)
$AUC_{0\text{-}\tau}$ (ng·hr/mL)	Area under the concentration-time curve over the dosing interval τ (24 hours), calculated by Linear Up/Log Down Trapezoidal Method
CL/F (L/hr)	Apparent total clearance calculated as CL/F =Dose/AUC0-τ

Pharmacokinetic parameters for dolutegravir in Part 3 on Day 7 and Day 28 include:

PK Parameter	Definition
C_{max} (ng/mL)	Maximum plasma concentration
T_{max} (hr)	Time to maximum plasma concentration
C_τ	Plasma concentration at the end of the dosing interval τ (24 hours)
$AUC_{0\text{-}\tau}$ (ng·hr/mL)	Area under the concentration-time curve over the dosing interval τ (24 hours), calculated by Linear Up/Log Down Trapezoidal Method
CL/F (L/hr)	Apparent total clearance calculated as CL/F =Dose/AUC0-τ

For each part, the estimated PK parameters will be summarized by analyte and dosing regimen with N, 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.

$AUC_{0-\tau}$ will be calculated with observed plasma concentrations from time zero to τ (actual time) without extrapolation or interpolation. The actual time for the predose (0 hours) will be replaced with zero. If observed plasma concentration at τ is BLQ, AUC_{0-last} will be used as $AUC_{0-\tau}$.

If the number of data points used to calculate λ_z (N_λ_z) is < 3 (not including C_{max}) 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-inf}, $t_{1/2,z}$, MRT, CL/F (Day 1), and V_z/F (Day 1), which are 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-inf}, $t_{1/2,z}$, MRT, CL/F (Day 1), and V_z/F (Day 1) will not be estimated. N_λ_z, and R^2 value for λ_z and AUC_{extr} will be listed.

If the AUC_{extr} is greater than 20%, then AUC_{0-inf}, MRT, CL/F (Day 1), and V_z/F (Day 1), which are derived using AUC_{0-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.

Plasma and urine PK parameters will be presented in the listings as follows:

- C_{max}: same precision as per the observed values
- C_τ: same precision as per the observed values
- T_{max}: two decimal places
- AUC_{0-last}, AUC_{0-inf}, and $AUC_{0-\tau}$: four significant figures
- AUC_{extr}: one decimal place
- λ_z: four decimal places
- N_λ_z: no decimal place
- R^2 value: three decimal places
- MRT, $t_{1/2,z}$, CL/F, V_z/F, Aeu_{x-y}, Feu_{x-y}, and CL_R: three significant figures

Descriptive statistics will be presented as follows:

- N and n: no decimal place

- Arithmetic mean, geometric mean, SD, median, Min, and Max: same precision as per each PK parameter in the listing.
- CV% and Geometric CV%: one decimal place

8.3. Dose Proportionality Assessments

The dose proportionality of plasma PK parameters of S-648414 will be examined in Part 1 (all fasted groups), and in Part 2. In Part 1, dose proportionality will be assessed for plasma S-648414 C_{max}, $AUC_{0\text{-last}}$, and $AUC_{0\text{-inf}}$ using 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 95% CI using SAS Proc Reg from the power model.

Statistic values of PK parameters will be presented as follows:
- Intercept, slope, standard error, and 95%CIs: three significant figures

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 also be 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 presented.

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.

Statistic values of PK parameters will be presented as follows:
- Geometric least squares (LS) means: same precision as geometric mean in Section 8.2
- Mean ratios and 90% CIs: four significant figures

8.4. Dose Independency Assessments

Dose independence of plasma PK parameters of S-648414 will be examined between the treatment groups in Part 1 (all fasted groups) and Part 2. For S-648414 $t_{1/2,z}$, 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 for plasma S-648414 to S-648414 dose will be presented graphically.

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

Statistic values of PK parameters will be presented as follows:
- Geometric LS means: same precision as geometric mean in Section 8.2
- Mean ratios and 90% CIs: four decimal places

8.5. Food Effect Assessment

S-648414 plasma PK data following a single administration of S-648414 tablet will be compared between fasted and fed conditions. An ANOVA will be performed using SAS Proc Mixed for ln-transformed C_{max}, $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$, and $t_{1/2,z}$ for plasma 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 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) / (PK in the fasted state)

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 $t_{1/2,z}$ for plasma S-648414 between the fasted state and the fed state will be presented graphically.

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.

Statistic values of PK parameters will be presented as follows:
- Geometric LS means: same precision as geometric mean in Section 8.2
- Mean ratios and 90% CIs: four decimal places

8.6. Accumulation Assessment

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.

Statistic values of PK parameters will be presented as follows:
- Geometric LS means: same precision as geometric mean in Section 8.2
- Mean ratios and 90% CIs: four decimal places.

8.7. Effect of S-648414 on the Pharmacokinetics of Midazolam

When data are available, the effect of S-648414 on the plasma PK of MDZ 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 MDZ. 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 MDZ coadministered with S-648414 and MDZ 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:
(MDZ coadministered with S-648414) / (MDZ 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 MDZ are completely contained within the range of 0.8000 to 1.2500. The comparison of C_{max}, T_{max}, $AUC_{0\text{-last}}$, and $AUC_{0\text{-inf}}$ between MDZ alone and MDZ coadministered with S-648414 will be presented graphically.

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

Statistic values of PK parameters will be presented as follows:
- Geometric LS means: same precision as geometric mean in Section 8.2
- Mean ratios and 90% CIs: four decimal places

8.8. Effect of S-648414 on the Pharmacokinetics of Dolutegravir

When data are available, the effect of S-648414 on the plasma 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% 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 presented graphically.

The effect of S-648414 on the plasma 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.

Statistic values of PK parameters will be presented as follows:
- Geometric LS means: same precision as geometric mean in Section 8.2
- Mean ratios and 90% CIs: four decimal places

8.9. Effect of Dolutegravir on the Pharmacokinetics of S-648414

When data are available, the effect of dolutegravir on the plasma PK of S-648414 will be assessed. An ANOVA will be performed using SAS Proc Mixed for ln-transformed C_{max}, C_{τ}, and $AUC_{0-\tau}$ 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 $AUC_{0-\tau}$. 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% C_{max}, C_{τ}, and $AUC_{0-\tau}$ of dolutegravir are completely contained within the range of 0.8000 to 1.2500. The comparison of C_{max}, C_{τ}, and $AUC_{0-\tau}$ between S-648414 alone and S-648414 coadministered with dolutegravir will be presented graphically.

The effect of dolutegravir on the plasma 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.

Statistic values of PK parameters will be presented as follows:
- Geometric LS means: same precision as geometric mean in Section 8.2
- Mean ratios and 90% CIs: four decimal places

9. Safety Analysis

9.1. Adverse Events

An AE is any untoward, undesired, or unplanned event in the form of signs, symptoms, disease, or laboratory or physiologic observations occurring in a person given a study intervention in a clinical study. The event does not need to be causally related to the study intervention or clinical study.

A treatment-emergent AE (TEAE) is defined as any event not present before exposure to any of study interventions ((S-648414, Midazolam, Dolutegravir, or Placebo) or any event already present that worsens in intensity or frequency after exposure to study intervention. A TEAE will be summarized to a given treatment if the event onset/worsening occurs any time after the dose in that period and before the dose in the next period.

Adverse Events will be coded by system organ class (SOC) and preferred term (PT) according to MedDRA, version to be delineated in the CSR.

All summary tables will include number and percentage of subjects and number of events. For the number of AEs, each occurrence will be counted once. In the summary tables where both SOC and PT are presented, AEs will be sorted in descending order of frequency of SOC based on the total of all treatment groups, and then alphabetically. Within each SOC, AEs will be sorted in descending order of frequency of preferred terms based on the total of all treatment groups, and then alphabetically. A subject with 2 or more AEs within the same level of summarization will be counted only once in that level using the most extreme incident (for the severity table) or the most related (for the relationship to study intervention table). Percentages will be based upon the number of subjects in the safety population by treatment group.

9.1.1. Incidence of Adverse Events

An overview of AEs will be presented by treatment group and overall for each part, including number and percentage of subjects with any:

- TEAE
- Treatment-related TEAE (S-648414 or placebo related, MDZ related, and Dolutegravir related)
- Any TEAE with Severity Grade 2-4
- Any TEAE with Severity Grade 3-4
- Gastrointestinal (GI) AE
- Ocular AE
- SAE
- Treatment-related SAE (S-648414 or placebo related, MDZ related, and Dolutegravir related)
- TEAE leading to study discontinuation
- TEAE leading to study intervention (S-648414 or placebo related, MDZ related, and Dolutegravir related) discontinuation
- Death

All TEAEs will be presented in a summary table by treatment and total for each SOC and PT.

All AEs recorded in the eCRF will be presented in a data listing.

9.1.2. Relationship of Adverse Events to Study Intervention

The relationship of AE to study intervention (S-648414 or placebo, MDZ, and Dolutegravir separately) will be classified as Not Related or Related to each study intervention by the Investigator.

Treatment-emergent AEs will be summarized by SOC, PT, and relationship to study intervention, and presented by treatment group and overall for each part. If there are any missing relationship information, a missing category will be added to the summary table. At each level of subject summarization, a subject is classified according to the closest relationship if the subject reported one or more events.

S-648414 or placebo related TEAEs, Midazolam related TEAEs, and Dolutegravir related TEAEs will be presented in data listings.

9.1.3. Severity of Adverse Event

The severity of AEs will be classified as Mild, Moderate, Severe, and Potentially life-threatening by the Investigator.

Treatment-emergent AEs will be summarized by SOC, PT, and severity, and presented by treatment group and overall for each part. Moreover, Treatment-related AEs will be summarized by SOC, PT, and severity, and presented by treatment group and overall for each part in a separate summary table. If there are any missing severity information, a Missing category will be added in summary table. At each level of subject summarization, a subject is classified according to the highest severity if the subject reported one or more events.

9.1.4. Serious Adverse Events

An SAE is an AE that:

- Results in death
- Is life threatening
- Requires inpatient hospitalization or prolongation of an existing hospitalization
- Results in a persistent disability/incapacity
- Results in a congenital anomaly or birth defect

Additionally, 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.

All SAEs will be completely followed for information to definitively obtain SAE status.

All SAEs will be presented in a summary table by treatment and total for each SOC and PT. All SAEs will be presented in a data listing.

9.1.5. Adverse Events of Special Interest

Adverse events of special interest include GI AEs and ocular AEs. Gastrointestinal AEs includes nausea, diarrhea or loose stools, abdominal pain/ abdominal discomfort/ stomach ache, and vomiting. If a GI AE occurs, the electronic data capture system will generate an additional case report form to collect further information regarding the event. Ocular AEs include iritis, dry eye, eye pain, and abnormal sensation in eye.

All GI AEs and ocular AEs will be presented in summary tables by treatment and total for each SOC and PT. All GI AEs and ocular AEs will be presented in data listings.

9.1.6. Adverse Events Leading to Study Discontinuation

Treatment-emergent AEs leading to study discontinuation will be summarized by SOC and PT, and presented by treatment group and overall for each part in a summary table. At each level of subject summarization, a subject is classified according to the highest severity if the subject reported one or more events.

9.2. Clinical Laboratory Evaluations

The protocol-required safety laboratory assessments (See Table 9.2.1) will be analyzed by [redacted]. Blood and urine samples will be collected at the time points indicated in the schedule of assessments (Section 12.1).

Table 9.2.1 Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	**Parameters**		
Hematology	Platelet count	RBC indices:	WBC count with differential:
	RBC count	MCV	Neutrophils
	Hemoglobin	MCH	Lymphocytes
	Hematocrit	%Reticulocytes	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 Pregnancy Test (only for females who are not diagnosed as postmenopausal.) • 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; Institutional Review Board (IRB) / Independent Ethics Committee (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 Protocol 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.

Abnormal clinical laboratory values will be flagged as either high or low (or normal or abnormal) based on the reference ranges for each laboratory parameter. The investigator will determine whether any of the abnormally high or low results are clinically significant or not clinically significant.

Actual results and changes from baseline values for hematology, clinical chemistry, and routine urinalysis tests will be summarized by visit and treatment group for each part.

Shift from baseline in hematology, clinical chemistry, and routine urinalysis tests results relative to the reference range will be summarized by visit and treatment group by each part, using the frequency count and percentage of subjects in each category.

All laboratory data will be presented in data listings.

9.3. Vital Sign Measurements

Vital sign measurements will include body temperature, pulse rate, respiratory rate, and blood pressure. The average of 3 consecutive readings for blood pressure will be displayed in summaries and all of the 3 readings will be displayed in listings. Vital signs will be measured at the time points indicated in the schedule of assessments (Section 12.1).

The investigator will determine if any of the vital sign measurements are clinically significant or not clinically significant.

Actual results and changes from baseline values for vital sign data will be summarized by visit and treatment group for each part.

All vital sign and weight/BMI data will be presented in a data listing.

9.4. Electrocardiogram

A triplicate 12-lead ECG for safety assessment will be obtained as outlined in the schedule of assessments (Section 12.1).

The investigator will determine whether any of the 12-lead ECG results are clinically significant or not clinically significant. Findings of 12-lead ECG will be classified as either “normal”, “abnormal, not clinically significant”, or “abnormal, clinically significant”.

For interpretation of triplicate 12-lead ECGs, baseline will be defined as the best case of the last non-missing triplicate measurements (including repeated and unscheduled measurements) before the start of first study intervention administration. For calculation of post-dose summary statistics, the worst case of the non-missing triplicate measurements will be used. The order of interpretation for 12-lead ECGs (best to worst case): Normal; Abnormal, Not Clinically Significant; Abnormal, Clinically Significant.

The shift from baseline of categorical investigator interpretation will be summarized at each visit by treatment group for each part for the safety population.

The investigator’s interpretation will be presented in a data listing for the safety population.

Cardiodynamic Electrocardiogram evaluations extracted from Holter recording will be in a separate SAP by another vendor.

9.5. Ophthalmological Examinations

Ophthalmological examinations performed in Parts 1 and 2 will include fundoscopy, tonometry (intraocular pressure), and a visual acuity test. Assessments will be carried out by an opthalmologist at the specified time points in the schedule of assessments (Section 12.1).

Ophthalmological examinations results will be presented in a data listing.

9.6. Physical Examination

Complete physical examinations and symptom-focused physical examinations relevant to the study participant's current condition will be performed as clinically indicated at visits specified in the schedule of assessments (Section 12.1).

The shift from baseline of categorical interpretation (normal, abnormal – not clinically significant, abnormal – clinically significant) will be summarized at each visit by treatment group for each part.

Physical examination results will be presented in a data listing.

9.7. Liver Events

When any safety test result meets the management and discontinuation criteria for liver function abnormalities (Protocol Section **Error! Reference source not found.** and Protocol Section **Error! Reference source not found.** [Appendix 6]), the results of further assessments and required follow-up should be recorded in the Liver Event Form.

Abnormal Liver Chemistry Criteria will be summarized by treatment group for each part for the safety population. Liver Events data will be presented in a data listing.

9.8. Biomarkers Sample Collection

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 (see Table 3.1.2.1). The study plans and results will be reported separately.

10. Interim Analysis

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 Protocol 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.

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).

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

11. Change in Planned Analysis

In Protocol, the enrolled population is defined as all participants who sign the ICF; The safety population is defined as all participants randomly assigned to study intervention and who take at least 1 dose of study intervention.

In the SAP for Part 3: the enrolled population will include all participants who sign the ICF and passed screening; The safety population will include all participants who take at least 1 dose of study intervention.

12. Appendix

12.1. Schedule of Assessments

Table 12.1.1: Schedule of Activities for Part 1

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
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	←							→
Concomitant medication monitoring	←							→
Special situations (abuse, misuse, overdose, or			←					→

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
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.

Table 12.1.2: Schedule of Activities for Part 2

Procedure	Screening Day	Admission Day	Confinement in CRU (Day)[a]																			Discharge from CRU (Day)	Follow-up or Early Termination Visit (Day)
	−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]	↔																							
Liver events collection	↔																							
Concomitant medication monitoring	↔																							

	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**
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.

Table 12.1.3.1: Schedule of Activities – Part 3, Period 1, Groups I and J

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)
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	←										→
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										

	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)**
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).

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.

	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)**

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.

Table 12.1.3.2: Schedule of Activities – Part 3, Period 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]	⟷																
Liver events collection	⟷																
Concomitant medication monitoring	⟷																

	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**
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.

12.2. 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.